1987

The effect of a balanced amino acid infusion on hepatic regeneration in rats

Robert Arthur Anstadt
University of the Pacific

The Effect of a Balanced Amino Acid Infusion on Hepatic Regeneration in Rats

A Thesis

Presented to

the Faculty of the Graduate School

University of the Pacific

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Robert A. Anstadt

April 1987

Abstract

The efficacy of a balanced amino acid infusion on recovery from temporary hepatic insufficiency and its effect on plasma ammonia levels in partially hepatectomized rats were studied.

Polyethylene implant buttons were developed to secure animals to the infusion apparatus. Rats under went jugular vein cannulation, and 70% partial hepatectomy. Test solutions were continuously infused (30 ml/d). Plasma samples were taken through the jugular vein cannula and analyzed for bilirubin concentration, ammonia concentration, LDH activity, and GPT activity.

LDH and bilirubin results were inconclusive. Results from GPT activity assays suggest that both Freamine III and the balanced formula provided injured hepatocytes with better nutrition than saline infusions and ad libitum food consumption. Ammonia assay results suggest that the balanced formula was more effective than Freamine in maintaining plasma ammonia levels in the normal range after partial hepatectomy.

Acknowledgements

The author would like to express his appreciation to:

Dr. Francis W. Sayre for his knowledge, guidance, friendship, and enthusiasm for this project which led me through the discouraging moments.

Dr. Anne Funkhouser for her time and helpful suggestions throughout this project, and her help with the animals at the beginning of this project.

Dr. John Brown for his time and assistance with this and other projects.

Sandy Mc Nett-McGowan for her help preparing the illustrations.

Ron Stauffer and Kendall Mc Gaw Inc. for their generous donation of Hyperlyte® and Freamine® solutions used in this study.

The many undergraduate students who helped me with the preliminary work.

My parents for providing me with the foundation making my education possible.

Finally, to Leigh for her continuing patience, support, and love, during my long quest to see this project to its end.

Table of Contents

Contents (Cont.)

Tables

Figures

Introduction

Over 150 years ago it was discovered that dogs fed a diet free of nitrogen would die prematurely, whereas this early death could be prevented by adding protein to this diet. Dietary proteins in their native form were not really what was saving the animals from their early demise, however since proteins as such cannot be absorbed by the digestive tract. Proteins must be broken down into their constituent amino acids before intestinal absorbtion can occur.

Of all amino acids in existence, 20 are shared by all living organisms. A few others are not found in proteins but play important physiologic roles. Some proteins also contain amino acids other than the 20 common ones. Amino acids pass through the intestine into the portal circulation in free form. Thus portal vein amino acid concentrations are much greater than systemic circulatory concentrations. Individual amino acids levels in the portal circulation do not reflect dietary protein amino acid composition since some endogenous amino acids from proteins secreted into the digestive tract are also absorbed. The amount of endogenous protein reabsorbed in the intestine has been estimated to be from 140 to 350 g/d.[1,2]

Plasma amino acid concentrations depend on the balance between addition of amino acids through intestinal absorbtion, tissue breakdown, and amino acid synthesis; and removal by protein synthesis, other metabolic processes and

excretion. Intestinal digestion of proteins is the rate limiting step in the uptake of free amino acids which are quickly absorbed. Increased amounts of dietary proteins have little effect on short term fasting levels of proteins.[3] In a typical experiment to determine the fate of ingested protein, dogs were fed a large meat meal. After the amino acids were absorbed in the intestine and passed through the portal vein to the liver, it was found that 57% were converted to urea, 6% were used in the synthesis of plasma proteins, 23% left the liver as free amino acids, and 14% remained in the liver presumably to form hepatic proteins. Within 15 minutes of absorbtion, 85% of free amino acids have been removed from the circulation. The liver, pancreas, kidney and intestinal mucosa all have a rapid uptake of amino acids. In the brain uptake of amino acids is very selective. Uptake of leucine, lysine, and proline is slow and there is almost no uptake of glutamic acid. Uptake of amino acids by skeletal muscle is slower than many other tissues, but muscle maintains a free amino acid pool longer than other tissues. Muscle is the largest proportion of body mass and therefore can contribute the most amino acids to the rest of the body as needed.[2]

Protein synthesis and degradation are ongoing processes. Each protein has its own half-life with the mean survival of all body proteins being about 80 days. Total body protein turnover has been estimated to be at least 150

grams per day, 100 grams of which are supplied by the diet. Catabolism of body proteins fills remaining protein requirements.

Serious alterations of protein metabolism may occur after injury depending on severity.[4] Simple fractures, elective surgery with little loss of blood, and other minor injury do not greatly alter body composition.[5,6,7] Massive injury, severe volume reduction, infection or a combination of these are needed to evoke the typical metabolic response to injury.[5] Body fuels are rapidly mobilized after major trauma in excess of their demand.[8] Skeletal muscle supplies amino acids for hepatic gluconeogenesis, protein synthesis and wound repair; however, complete reutilization of amino acids does not occur and there is a loss of body nitrogen which is greater than losses produced in starvation without stress. There is also an increased oxidation of branched chain amino acids (BCAA) within skeletal muscles which suggest an inability of skeletal muscle to utilize non-protein energy substrates.[4,9]

Many studies have documented a post-trauma increase of whole body protein turnover which can be from 75 to 150 grams nitrogen per day (300 to 600 g lean body mass).[7,10,11] Whether increased turnover is due to a reduction of whole body protein synthesis or an increase in whole body protein breakdown has not been determined, and may be dependent on the type of injury.[11] The effect of trauma on the

metabolism of individual tissues has also not been fully determined. There is some agreement that there is an increased synthesis of hepatic non-secretory proteins, but results on protein synthesis by muscle are inconclusive.[11] Protein loss with trauma can be great enough to impair wound repair, resistance to infections, and synthesis of enzymes and plasma proteins.[9] There has been a great deal of interest in trying to correct a patient's negative nitrogen balance[a] to bring about a more speedy recovery. Glucose infusions have been used to spare body protein, but can be detrimental to the patient over time if used as the sole source of protein sparing therapy without any intake of alpha-nitrogen compounds. Glucose spares protein by decreasing protein breakdown and causing uptake of free plasma amino acids by skeletal muscle presumably through the hormonal effects of insulin. The end result of glucose therapy can be to cause a decreased supply of essential amino acids. Glucose infusions have been shown to limit albumin synthesis after trauma,[6] while infusions of mixtures of essential and non-essential amino acids increase albumin synthesis.[12] Amino acid infusions are effective in maintaining essential amino acids at concentrations that can support protein synthesis.[6]

---

a. Nitrogen balance refers to the relative amounts of nitrogen being absorbed and excreted by the body. Thus nitrogen balance is negative if the amount of nitrogen being excreted is greater than the amount being taken in.

Post-traumatic catabolism of skeletal muscle protein is approximately double the amount of whole body catabolism.[7] The branched chain amino acids, valine, leucine, and isoleucine are the only amino acids oxidized primarily extrahepatically.[13] Under normal conditions, glucose and pyruvate inhibit BCAA oxidation.[14] Starvation, stress, diabetes and other conditions associated with muscle wasting stimulate BCAA oxidation.[14,15] Several regulatory functions have been ascribed to BCAA including promotion of protein synthesis, inhibition of protein degradation, and stimulation of alanine and glutamine synthesis [13,14] Which, if any, of the BCAA individually is regulatory has not been determined. Leucine has been implicated in several studies[14,16] while valine has in others.[10] These observations have led to experiments testing the protein sparing ability of BCAA infusions. Infusions containing a high proportion of BCAA have shown good results in bringing about a positive nitrogen balance after trauma in man, and experimental animals.[17,18,19,20] The ability of BCAA infusion to improve nitrogen balance increases with their proportion in solution until a maximum 32% (weight/weight of alpha nitrogen compounds) is reached. Beyond this percentage, lack of other amino acids hinders optimal protein synthesis.[15]

Amino acids cannot be stored by the body, and there is little accumulation of free amino acids by tissues; there-

fore, rapid utilization of available amino acids must occur for optimum protein synthesis.[21] All constituent amino acids of a protein must also be simultaneously present for its synthesis. Amino acid availability for all body cells is supplied in the plasma, which maintains their concentration within narrow limits. These relative plasma concentrations of amino acids have been referred to as the plasma balance of amino acids.[22] Nutritional studies using cell free systems (derived from mouse liver)[22] multi-celled organisms[23] (nematodes in axenic culture) and perfused heart[24] have demonstrated the importance of amino acid balance on protein synthesis.

The objectives of this study were to evaluate the efficacy of a balanced amino acid solution on the recovery from temporary liver insufficiency produced by partial hepatectomy in rats. A complication of parenteral nutrition therapy sometimes seen in patients with liver insufficiency is hyperammonemia.[25] A second objective of this study was to determine the effect of balanced amino acid infusion on plasma ammonia concentrations.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 100 g were obtained from Simonsen Laboratories, Gilroy California. Purina rat chow and water were provided ad libitum. Rats were weighed daily, and allowed to acclimate for a minimum of one week.

Animals were fasted over night prior to surgery after reaching a minimum weight of 150 g.

Jugular Vein Catheterization and Button Implantation

The method for jugular vein cannulation was a modification of Bakar et. al.[26]

Cannula preparation. A 56 cm length of medical grade silicon rubber tubing (Silastic, Dow Corning Co.) was wrapped twice and tied with a 15 cm length of 4-0 silk suture 28 mm from one end of the tubing. Suture material was tied tight enough to avoid easy slippage, yet not so tight as to seal the tubing lumen. The knot and wrapped portion of suture material was coated with a thin layer of silicone rubber aquarium sealant (Dow Corning Co.) and allowed to cure at least 24 hours. Cannulas were sterilized by soaking in 0.5% benzalkonium chloride for at least 1 hour before implantation (Fig. 1a, p. 36).

Cannula needle preparation. The hubs were removed from 20g x 38mm and 23g x 15 mm needles. The cut ends of both needles were then deburred and squared with a very fine file. The pointed end of the 23g needle was then honed with a whetstone until about 2 to 3 mm would fit snugly and securely in the squared end of the 13g needle (Figs. 1b and 1c, p. 36). A bend was gently made in the 20g needle approximately 15mm from the pointed end.

Polypropylene implant button fabrication (model 1). Implant "buttons" were made from polypropylene pipette tips for the MLA applicator pipette, 0 to 200 µl size. Approximately half of the pipette tip from the base end was melted over a bunsen burner flame, taking care to avoid igniting the polypropylene. The molten end was placed on a glass slide and flattened. After cooling and hardening, a hole for catheter passage (approximately 2 mm in diameter) was made in the center of the base by passing a red-hot wire through it. Three other holes (approximately 1 mm in diameter) for passage of sutures were placed at equal distances around the central hole. The top 7.0 mm, as measured from the tip of the pipette, was then cut off. Length removed is dependant on catheter and spring catheter shield diameters. All rough flashing was trimmed from the button surface (Fig. 2, p. 38).

Polypropylene implant button fabrication (model 2). A 0-200 µl size pipette tip for the MLA applicator was used as the main piece for implant button construction. The pipette tip is conical in shape, with a base of about 7mm and a height of about 48mm. Equidistant around the circumference are three projections about 8mm long running lengthwise. Using a red hot needle, a 1 mm hole was placed on both sides of these projections, 15 mm from the base of the pipette tip. A piece of copper wire about 0.5 mm in diameter was threaded through a hole on one side of a

projection and brought out on the other side. This was done for each of the three projections. The ends of each wire were twisted together with the ends of adjacent wires, and cut to a length of 10 mm. A coat of silicon aquarium sealant was applied to the wire tips and allowed to dry for at least 24 hours. Fabrication was completed by removing 13mm of the base and 7 mm of the tip (Fig. 3, p. 40).

Surgical implantation. Rats were fasted 24 hours prior to surgery. Methohexital sodium (40 mg/kg i.p.) was used to induce anesthesia followed by pentobarbital (40 mg/kg i.p.) 4 to 7 minutes later to insure prolonged anesthesia. Lidocaine (1%) was given subcutaneously as needed to control reflex twitching during implantation. Animals were shaved in the neck and midscapular regions after induction of anesthesia. (Shaving of animals is not required but makes the procedure easier and is recommended.) Whiskers were also clipped to reduce the animals tendency to move around later in the experiment.

Animals were restrained in the supine position with the head towards the surgeon. In younger rats the jugular vein can be seen pulsing under the skin, but may be obscured by fat in older animals. A skin incision (1 to 1.5 cm long) was made over the right jugular vein parallel to the animal's midline. In animals where the jugular vein cannot be seen, the incision was made 1 to 2 cm lateral to the midline. The jugular vein was isolated by blunt dissec-

tion. Care must be taken at this step because excess jugular vein manipulation can cause its constriction, making cannulation more difficult. (If constriction occurs the jugular vein will dilate after a few minutes.) After isolating the vein, the cephalic end was grasped with fine forceps and a small lancet was used to cut a small opening in the vein caudal to the forceps. The implantation needle was inserted through the hole and passed along the jugular vein. The needle was then passed out of the vein through the deltoid muscle, to help control bleeding (Fig. 4a, p. 42). Any blood clots formed in the needle during passage through the jugular vein were removed by flushing the cannula assembly with heparinized saline. The needle was carefully disconnected from the cannula and the cannula flushed with more heparinized saline to remove any air bubbles. The cannula was pulled slowly back until it lay in the jugular vein and then advanced towards the heart until the knot reached the vein. Cannula blood flow was checked by withdrawing the syringe plunger. If no blood could be aspirated, the cannula was pulled back until blood flow could be obtained. Otherwise the cannula was flushed with enough saline to just clear the cannula of blood. Cannulas were secured by suturing to the underlying musculature. The cannula was clamped with a hemostat and removed from the syringe. A wire plug was inserted into the end of the cannula and the hemostat removed.

The animal was turned over and a 2 to 3 centimeter longitudinal skin incision was made in the animal's midscapular region. Skin was freed from underlying facia for a distance of 1.5 cm in all directions from the incision. A hub-less 16 gauge needle was passed subcutaneously from the neck incision to the midscapular incision (Fig. 4b, p. 42). The plugged cannula was threaded through the needle, and exteriorized through the mid-scapular incision. The needle was removed through the midscapular incision leaving the cannula in place. A polypropylene button was threaded over the cannula and positioned under the skin on the animals back, and then sutured to the musculature and skin with 4-0 silk. The syringe was reconnected to the cannula and blood flow was checked again. If no blood flow was obtained, the button was positioned until blood flow started. Once sufficient blood flow was obtained the back wound was closed with 4-0 silk sutures leaving the button tip and cannula protruding (Fig. 4c, p. 42). The neck incision was closed using 5-0 silk sutures. The cannula was threaded through the protection spring, and the spring screwed onto the tip of the button (Fig. 5, p. 44).

All animals were housed individually and provided rat chow ad libitum after jugular vein cannulation. No oral water was allowed.

Preparation of Solutions

Balanced amino acid solution. All amino acids except glutamic acid were weighed and placed in an Erlenmeyer flask. Double distilled water was added and the amino acids were dissolved to form a solution of approximately 1.5 times the desired final concentration. Glutamic acid was weighed and dissolved in double distilled water adjusted to pH = 10 with saturated NaOH solution. The final concentration of the glutamic acid stock solution was ten times the required concentration of the final balanced solution. The required amount of concentrated glutamate solution was then added to the concentrated amino acid solution. To this solution Hyperlyte brand of electrolytes were added (25 ml Hyperlyte/ 1L balanced amino acid solution). Required amounts of water-soluble vitamin solutions were added (Table 1, p.45). The pH was adjusted to approximately 6.5 with glacial acetic acid, and then diluted to its proper concentration (Tables 2, p. 46, and 3, p. 47).

Half-normal saline. For each liter of half normal saline, 4.25g NaCl was dissolved in double distilled water. This solution was then diluted to a final volume of 1.0 liter.

Electrolyte solution. For each liter of electrolyte solution, 25 ml of Hyperlyte® was added to double distilled water, and diluted to the proper volume.

Freamine III. One bottle of Hyperlyte® (25 ml) was aseptically added to each liter bottle of Freamine® III, and was then ready to use.

Sterilization of solutions. All ingredients were mixed thoroughly and placed in a stainless steel container attached to a Millipore 90mm diameter filter (0.22 micron pore size). Solutions were filtered into a sterile Erlenmeyer flask using ultra-pure nitrogen as the propellant. Solutions were poured into sterile infusion flasks under a laminar air flow hood, and plugged with a sterile rubber stopper after filtration.

Partial Hepatectomy

One-half normal saline was infused continuously (30 ml/d) via the jugular vein for 72 hours following cannulation. Animals were provided with rat chow ad libitum during the first 48 hour of this period, and were fasted during the next 24 hours prior to partial hepatectomy.

Each rat was anesthetized with methohexital sodium injected through the jugular cannula. The dose of methohexital was titrated individually for each animal by observing response to painful stimuli and breathing rate. Once an adequate plane of anesthesia had been reached, animal's were placed on their backs in a V shaped operating board constructed from half inch wire mesh. This was necessary because of the protruding implant button and spring apparatus on each animal's back. The springs were

folded back under the animals with the swivels and tails facing the investigator. Additional anesthetic could easily be given as needed during surgery with the animals in this position.

A midline ventral abdominal skin incision was made from just above the xiphoid process to about half way down the abdomen (Fig. 6a, p. 49). An incision was then made through the abdominal muscles from about mid way on the abdomen to the xiphoid process. The falciform ligament which extends along the midline from the convex face of the liver to the diaphragm was cut. A bolster was then placed under the animal to arch his back, and a piece of gauze was placed at the caudal end of the incision. Using finger tips the left lateral lobe of the liver was gently pulled part way out of the abdominal cavity, and the suspensory ligament which connects the left lateral lobe to the hepatic portal vein was cut. The median lobe was "popped" out of the abdominal cavity by squeezing and pushing down on the abdomen just below the rib cage with the left hand and maintaining gentle traction on the left lateral lobe with the fingers of the right hand (Figs. 6b and 6c). Traction was maintained on both exteriorized lobes with the left hand while the right hand put a ligature around them and their blood vessels. The ligature was tied (Fig. 6d), and the lobes were placed on gauze pads, Several cuts were made in each lobe so blood would be absorbed by the gauze, and not drip into the

abdominal cavity when the lobes were severed (Fig. 6e). The lobes were then transected and removed. Any excess ligature material was also removed. Muscular incisions were closed with a continuous suture using 4-0 silk. Skin incisions were closed with 9mm stainless steel wound clips. After surgery animals were returned to their cage and an infusion of half-normal saline was started until animals regained consciousness, whereupon a test solution was started.

Experimental Groups

Animals were divided into 5 groups following partial hepatectomy . Group IA was provided with rat chow ad libitum, and infused with half-normal saline. Group IB was provided with rat chow ad libitum and infused with Hyperlyte® (Kendall-Mc Gaw Inc.) electrolyte solution. Group II was provided with oral glucose pellets ad libitum and infused with Freamine®III. Group III was provided with oral glucose pellets ad libitum, and infused with the balanced amino acid solution. Group IV had a sham partial hepatectomy. Food and infusion for this group was the same as for Group IB. The infusion rate for all solutions was 30 ml/day (Table 4, p. 50).

Sample Collection

A 1 ml syringe with a blunt 22g needle was filled with 0.2 ml of sodium heparin. In order to prevent air from entering the cannula, the distal end of the cannula was clamped with a fine hemostat prior to detaching the cannula

from the swivel. The cannula was detached from the swivel and the blunt syringe needle was inserted into the cannula, and the clamp released. A 0.8 ml of blood was then aspirated into the syringe. The syringe was held vertically with the needle pointing downward to prevent dilution of blood by the heparin solution during blood aspiration. The cannula was again clamped and the needle removed after aspiration. The blood sample was injected into three sodium heparin treated glass capillary tubes and one plain glass capillary tube (0.2 ml total). Capillary tubes were sealed with Seal Eze and centrifuged for 5 minutes in a hematocrit centrifuge. Immediately after centrifugation, plasma was separated from red cells and placed into Dispo beakers, covered with Parafilm and placed on ice. Any remaining blood and heparin in the syringe was re-injected through the cannula into the animal.

Samples were collected at the following times:

1. 48 hours after cannulation (24 hours prior to partial hepatectomy)
2. 24 hours after partial hepatectomy
3. 48 hours after partial hepatectomy
4. 96 hours after partial hepatectomy
5. 144 hours after partial hepatectomy
6. 192 hours after partial hepatectomy.

Assays

Initial assays on group IA were done in triplicate. All other group assays were done singly. All spectrophotometric measurements were done on a Carl Zeiss spectrophotometer model PMQ II.

Lactate Dehydrgenase (LDH) Assay

Materials. All chemicals for the LDH assay were obtained from Sigma Chemical company, St. Louis, MO.

Pyruvate substrate, Stock No. 500L-1, Lot No. 123F-6110,

0.75 mmol/l sodium pyruvate in a buffered solution.

Color reagent, Stock No. 505-2, Lot No. 102F-6060,

2,4-dinitrophenylhydrazine, 20 mg/100 ml, in 1.0 N HCl

NADH, Stock No. 340-101, Lot No. 612-F4,

NADH 1.0 mg (dissolved in pyruvate solution prior to assay).

0.4 N NaOH

Method. In a small beaker, 10 µl of serum was mixed with 50 µl of double distilled water. The substrate solution was made by adding 1.0 ml of the pyruvate solution to a vial of NADH and gently mixing. One hundred µl of this solution was then placed in a 10 x 50 mm test tube and put into a 37° water bath for a few minutes to equilibrate temperatures. After equilibration, 10 µl of diluted serum was added to the substrate in the test tube and placed in the water bath for 30 minutes. The tube was removed from the water bath and 100 µl of color reagent was added, and

mixed. After 20 minutes, 1.0 ml of 0.4 N NaOH was added, and mixed. Absorbance was read at 505 nm after five minutes. LDH activity was then calculated from a standard curve (Fig. 7, p. 52).

LDH standard curve. (See table 5, p. 53 for contents of each tube for the standard curve). Approximately 20 minutes after the addition of the color reagent to the tubes, 1.0 ml of 0.4 N NaOH was added to each tube. Absorbance was read at 505 nm five minutes later.

Ammonia Assay

Materials. All reagents were obtained from Sigma.

Sigma Ammonia Reagent No. 170-3 Lot No. 35F-6110

Sigma L-Glutamate Dehydrogenase Solution, No. 170-4 Lot No.637-F5

Sigma Ammonia Control Solution, No. 170-5, Lot No. 15F-6137

Method. The ammonia assay is a modified version of Sigma's ammonia assay. Each ammonia assay was carried out in a micro-volume quartz cuvette. In a cuvette, 300 µl of ammonia reagent was gently mixed with 20 µl of plasma. Initial absorbance was read at 340 nm. After standing at room temperature for five minutes. Glutamate dehydrogenase (2.0 µl) was added to the cuvette, gently mixed, and allowed to sit at room temperature for five minutes. Final absorbance was read at 340 nm. A blank and control were done for each group of assays. Water and control solutions were

substituted for plasma in the preparation of the blank and control respectively.

Ammonia concentration was found by subtracting the final absorbance from the initial absorbance, and multiplying by a factor of 13.6. Ammonia concentration of the blank was subtracted from the plasma value.

Bilirubin Assay

Materials. All chemicals were obtained from Sigma.

Caffeine reagent. Stock No. 605-2, Lot No. 24F-6090, Caffeine, 25g/L, and Sodium benzoate 38g/L in sodium acetate solution.

Alkaline tartrate, Stock No. 605-3, Lot No. 24F-6089, Sodium potassium tartrate, 350 g/L, in sodium hydroxide solution.

Hydrochloric acid, Stock No. 945-50, Lot No. 63F-6115, 0.05 N HCl.

Diazo reagent, Stock No. 605-7, Lot No. 24F-6087, 75 µmol sulfanilic acid, and 6.6 mol sodium nitrite. (Reconstituted with 6 ml HCl 0.05 N prior to use.)

Cysteine reagent, Stock No. 605-6, Lot No. 24F-6088, Cysteine hydrochloride, 100 mg in 10.5 ml water.

Method. (See table 6, p. 54 for contents of each tube).

Absorbance was read at 600 nm. within 30 minutes of addition of alkaline tartrate. Bilirubin concentration was calculated by multiplying absorbance by 44.

Glutamic Pyruvic Transaminase (GPT) Assay

Materials. All reagents were from Sigma.

Alpha-ketoglutarate substrate, Stock No. 505-51, Lot No. 43F-6111.

Color reagent (same as used in LDH assay).

0.4 N NaOH

Method. One hundred µl of alpha-ketoglutarate substrate was added to a 9 x 70 mm test tube, and placed in a 37° water bath for a few minutes to equilibrate temperature. After equilibration, 20 µl of plasma was added, and the tube was replaced in the 37° water bath. Exactly 30 minutes later, 100 µl of color reagent were added, mixed, and allowed to stand at room temperature for 20 minutes before adding 1.0 ml of 0.4 N NaOH. Color was allowed to develop for five minutes before being read at 505 nm. GTP activity was determined from the standard curve (Fig. 8, p. 56).

GPT standard curve. (See table 7, p. 57 for the contents of each tube used in constructing the standard curve).

Results

Animal Viability

The over all survival of animals varied considerably between groups, and somewhat within groups. Two animals in the Freamine group died on the last day of the experiment. All other animals in this group finished the experiment.

Animals infused with the electrolyte solution had the shortest mean survival time (mean = 8.67 days, s.d. = 2.05) The sham surgery and balanced formula groups had intermediate mean survival times (Table 8a, p.58).

A complete autopsy was not done on the animals and thus an exact cause of death cannot be determined. Post mortem examination yielded consistent findings which could have caused death of animals. A yellow colored "plaque" was found in the vena cava and atrium in 8 of the 10 animals which died prior to the finish of the experiment. Animals that finished the experiment did not have "plaque" occluding the heart. Only 1 animal which died showed signs of liver failure. This animal died 72 hours after partial hepatectomy and was being infused with the balanced formula. One remaining animal which did not finish the experiment accidentally asphyxiated itself while trying to escape from its cage.

Weights were measured just prior to cannulation and at the end of the experiment or time of death. Animals infused with the electrolyte solution had the greatest weight loss of all groups (mean = 88.99% of baseline). The sham surgery group had a mean = 95.71% of baseline. Average weights of the balanced group and the Freamine group was essentially equal at the start and finish of the experiment (Table 8b).

LDH Assay

Early experiments with saline infused controls did not show any change in serum LDH activity at any sampling time. As a result of this finding, this assay was not done on any other experimental group (Fig. 9, p. 60).

Hematocrit

There was a decrease in hematocrit after partial hepatectomy, with the lowest hematocrit values at 96 hours after the surgery in the Freamine and sham groups. The lowest hematocrit values for the balanced and electrolyte groups were at 144 hours and 48 hours respectively. Hematocrit did not continue to fall with repeated blood sampling. Hematocrit values for all groups were somewhat higher at the end of the experiment than values recorded during the previous two samplings. The sham surgery group had a final hematocrit equal to 103% of the initial, which was the highest value of all groups (Fig. 10, p. 62).

Direct Bilirubin

In this study direct bilirubin is bilirubin which is not bound to any plasma proteins. Direct plasma bilirubin levels for the Freamine and the balanced groups approximately doubled in the first 24 hours after partial hepatectomy then returned to normal in the following 72 hours. Bilirubin levels for the saline and sham control groups stayed approximately at baseline levels throughout the experiment. The initial bilirubin level for the sham

group (0.25 mg/dl) was lower than the saline (2.46 mg/dl) and balanced (1.35 mg/dl) groups ($p < 0.025$). The bilirubin level 24 hours after partial hepatectomy for the Framine group (3.64 mg/dl) was greater ($p < 0.01$) than the sham group (0.34 mg/dl). The balanced group bilirubin value (3.63 mg/dl) was greater ($p < 0.05$) than the saline group (2.17 mg/dl). The balanced group (1.14 mg/dl) was lower ($p < 0.025$) than the Freamine group (2.28 mg/dl) and lower ($p < 0.05$) than the saline group (2.22 mg/dl) 96 hours after partial hepatectomy. The Freamine group bilirubin value (1.58 mg/dl) was lower ($p < 0.025$) than the saline group (2.27 mg/dl) 144 hours after the partial hepatectomy. Any difference between the groups 48 hours and 192 hours after the partial hepatectomy could not be determined with any reliability due to the large standard deviation and/or small number of samples (Table 9, p. 65, and Fig. 11, p. 64).

Total Bilirubin

Total bilirubin in this study was the measurement of free plasma bilirubin and plasma protein bound bilirubin. Total bilirubin levels for the Freamine and sham groups peaked 24 hours after partial hepatectomy. The balanced group and electrolyte group peaked 48 hours after surgery. Values for the saline group remained relatively constant during the experiment. The initial bilirubin value of the saline group (2.29 mg/dl) was greater ($p < 0.05$) than any other group. The initial bilirubin value for the balanced

group (1.28 mg/dl) was greater ($p < 0.025$) than that of the sham group (0.33 mg/dl). Comparison between groups 24 hours after partial hepatectomy could not be made with any reliability due to the large standard deviations of the groups. 48 hours after partial hepatectomy, bilirubin value of the balanced group (6.25 mg/dl) was greater ($p < 0.25$) than the Freamine group (3.66 mg/dl). Both of these groups were also greater ($p < 0.05$) than the saline value and greater ($p < 0.1$ for the balanced group and $p < 0.025$ for the Freamine group) than the sham group. The Freamine group bilirubin level was still elevated above the saline group at 96 hours ($p < 0.025$) and 144 hours ($p < 0.005$). The balanced group bilirubin level (0.49 mg/dl) was also lower ($p < 0.005$) than the saline (2.40 mg/dl) and Freamine (2.58 mg/dl) groups at 144 hours. There was no significant difference between groups at 192 hours after partial hepatectomy (Table 10, p. 68, and Fig. 12, p. 67).

Plasma Ammonia Levels

Data from all groups were averaged together to find a baseline plasma ammonia concentration (mean = 2.97μg/ml, s.d. = 8.10). Plasma ammonia increased in the Freamine group 24 hours after partial hepatectomy (3.64 μg/ml), but decreased in the balanced (2.23 μg/ml) and sham (1.07μg/ml) groups. Ammonia levels fell below baseline values for all groups during the next 24 hours. Sham group data is not

reliable after the 48 hour sampling time because of difficulties in obtaining samples from a large enough population of animals. Determination of ammonia levels at the 96 hour sampling time was not possible because of technical difficulties with ammonia standards. Ammonia levels for the Freamine (1.77 μg/ml) and the balanced (0.96 μg/ml) groups were below baseline levels 144 hours after partial hepatectomy (Fig. 13, p. 70).

GPT Assay

The GPT activity increased for all groups after partial hepatectomy. The low baseline reading for the sham group was due to a bad NaOH reagent. Sham group GPT values were fairly constant and the lowest ($p < 0.005$) of any group during the experiment. GPT values for the electrolyte controls were the highest ($p < 0.005$) at all sampling times measured. Freamine and balanced groups were back to baseline levels by 96 hours after surgery. The Freamine group had the least increase in GPT activity of all partially hepatectomized groups. Saline group GPT activity (181.18 S.F.units) was greater ($p < 0.05$) than the balanced group (116.54 S.F.units) and ($p < 0.01$) the sham group (79.9 S.F.units) 24 hours after partial hepatectomy. Saline group GPT activity was still elevated above other groups 48 hours after partial hepatectomy. The Freamine group (11.35 S.F. units) had a lower ($p < 0.025$) GPT activity than the

balanced group (28.21 S.F. units) 96 hours after partial hepatectomy. No other statistically significant differences could be found in the remaining data (Table 11, p. 71, and Fig. 14, p. 73).

Discussion

The literature provides several methods for jugular vein cannulation. Many of these are essentially the same.[27,28,29] While these procedures were adequate, they sometimes took an inordinately long time. This was usually a result of failing to get the catheter in the vein on the first attempt, and having the vein constrict making subsequent attempts difficult. The method of Bakar and Niazi proved to be more efficient than other cannulation techniques tried in this study. The initial extra preparation time of this method was negated by the ease and speed with which this procedure could be carried out. With this method it was possible to have a 100% success rate.

If animals are to be cannulated for continuous infusion of any solution, then the cannula must be protected from the rat and its tendency to chew anything available. Stress induced by restraining cages precludes their use in long term studies such as this one. Two methods which allow animals freedom of movement and cannula protection are the use of a swivel-spring assembly connected to a harness/jacket or to an implant button. While all of these methods cause some stress to the animal, the implant button is the

least stressful.[30] Both of these methods were tried in this study. Jackets were hard to adjust to a proper fit. Even if they were adjusted properly there was an unacceptable escape rate. Jackets also had a tendency to cause head, neck, and forearm edema. Use of implant buttons caused no edema, and the escape rate was zero.

Jugular vein cannulation for continuous nutrient infusion in rats has been successfully done by a number of researchers. Rats totally fed by this method have been maintained for as long as 40 days.[28] Maintenance of cannula patency for blood sample collection is a different story. In this experiment, the longest time that any samples could be collected from any animal was 16 days after cannulation. This was achieved in only 10% of the population (Fig. 15, p. 75). A study comparing various cannulation sites and maintenance of patency had a 50% success rate on day 14.[31]

Animals in both the Freamine and balanced groups maintained weight, even after losing about 10 grams following partial hepatectomy. The sham and electrolyte control groups lost 4.25% and 11.01% respectively. These findings are not in agreement with reported values which were on the order of a 15% weight loss for all experimental groups.[10,13,15] the reason for this discrepancy is not known.

A somewhat distressing finding of this study was a high animal attrition rate. A factor common in many animals

which died, or were terminated because no blood samples could be obtained was the appearance of a yellow colored "plaque" in the right atrium and vena cava. It is assumed that this plaque is a thrombus formed of platelets. Thrombus formation is a well known complication of parenteral nutrition therapy occurring with a frequency of 6.5 to 21% [32] In this study a thrombus formed in the vena cava and atrium in 50% of all experimental animals. Of all animals which died before the end of the experiment, 64% had a thrombus. Three other animals (15%) were terminated prior to the end of the experiment due to cannula blockage by thrombi. The Freamine group had the lowest frequency of thrombus formation. All of the other groups had approximately the same frequency. The reason for these differences are not known. Factors known to be associated with thrombus formation include: endothelial damage during catheter insertion, a break in sterility or the presence of a foreign body. These factors really do not account for any difference between the Freamine and other groups since the same experimental methods were used in all groups. Thus it would be expected that thrombus formation should have occurred with an equal frequency in all groups. An unknown thrombogenic factor may have inadvertently been incorporated into infusion solutions as residue on glassware or remained in solution after distillation and filtration.

Extreme hypertonicity of the infusion solutions could be another possible reason for the overall higher rate of thrombus formation. Infusion of a hypertonic solution into a large bore vessel is usually not a problem due to rapid mixing and dilution of the infusate. Thus hypertonic solutions may be safely infused into the vena cava whereas the same solution infused into a peripheral vein would cause problems. In total parenteral nutrition of man, the ratio of area between the outside cannula diameter and the inside vein diameter is 0.20 to 0.25. In this study the ratio was 0.56. Thus it is possible that the solutions were too hypertonic for infusion into small diameter veins and atria of rats.

The relation between elevated plasma bilirubin levels producing jaundice and hepatic disease or injury has been established for some time. Normal plasma concentrations of bilirubin are 0.1 to 0.4 mg/dl for direct bilirubin and 0.1 to 1.3 mg/dl for total bilirubin in humans.[33] Plasma bilirubin concentrations for rats are also approximately 0.3 mg/dl depending on species.[34] Jaundice becomes clinically apparent at plasma concentrations above 2.0 mg/dl.[35] Carbon tetrachloride and other hepatotoxins have been reported to produce an increase in plasma bilirubin lasting 6 weeks after administration.[36] Regenerating liver shows a decreased ability to excrete bilirubin into the bile during the first 24 hours after partial hepatectomy.[37] The

maximal rate of bilirubin excretion has been shown to be depressed by 80% after 70% hepatectomy, which is a 20 to 30% greater decrease than expected.[38] Hepatic uptake of bilirubin has also been shown to decrease after partial hepatectomy returning to normal ranges 4 days later. The mechanism behind this decrease is not known but is thought to be a result of a decreased amount of organic anion binding protein in the immature hepatocyte plasma membrane.[39] Despite these findings several studies have been unable to document any increase in plasma levels of endogenous bilirubin after 70% partial hepatectomy.[40,41] An increase was reported after 80% sub-total hepatectomy.[40] In this study there was no increase of plasma bilirubin in sham, saline or electrolyte groups. A large increase was seen in the balanced group. Two animals in this group had a continual increase of their plasma bilirubin after partial hepatectomy. Neither animal finished the experiment and both had extensive yellow discoloration of their livers during post mortem examination. A slight increase was also seen in the Freamine group during the first 48 hours after partial hepatectomy. Bilirubin levels calculated for the saline, balanced and Freamine groups all were higher than the accepted normal range. Reasons for these high readings are not known and therefore cast some doubt on the reliability of the quantitative bilirubin results. However, plasma did become yellow colored after partial hepatectomy

in these groups indicating a qualitative presence of bilirubin.

Glutamic pyruvic transaminase (GPT) is found in high concentrations in liver and low concentrations in other tissues. GPT is released into the blood by the lysis of necrotic hepatocytes. In man plasma GPT concentrations range from 0 to 21 SF units/ml.[42] Rat plasma usually has GPT activity in the upper end of the human range.[34] Several studies have documented elevated plasma GPT levels following partial hepatectomy. Maximal levels occurred 12 hours after partial hepatectomy and gradually returned to normal ranges on the fourth post operative day. The degree of plasma GPT elevation was correlated with the amount of liver removed.[38,40] Results of this study are in general agreement with the above findings. A discrepancy with the above findings was the increased plasma GPT activity of the sham group after partial hepatectomy. GPT activity for this group remained above baseline values at all sampling times. Sham group data must be viewed with some cautionis since it is based on a small number of animals, especially after the 48 hour point. Both the Freamine and balanced groups had lower increases in plasma GPT activity than the saline group. This suggests that hyperalimentation infusions provided cells injured during surgery with better nutrition and thus prevented their death and subsequent release of GPT.

Normal plasma ammonia concentration in humans ranges from 80 to 110 g/dl.[35] A literature search failed to find the normal range of plasma ammonia in the rat. In this study, a baseline value of 297 g/dl was found. Rats infused with the balanced formula had plasma ammonia levels below baseline at all sampling times after partial hepatectomy, and they were also lower than levels in rats infused with Freamine III. This suggests that a balanced amino acid solution can be infused without causing hyperammonemia in rats with temporary hepatic insufficiency. However, the above needs to be viewed with some caution. A large standard deviation and/or a small population within each group made any statistical difference between groups questionable. Ammonia standards were also subject to occasional irregularities casting some doubt on the overall precision of some individual ammonia determinations.

Although ammonia results seem rather high, they are still lower than elevated plasma ammonia values recorded in serious hepatic disease which may exceed 1000 g/dl.[43] If the plasma ammonia levels are truly as high as the data suggest, one of several possibilities may explain why. Patients should be fasted at least 6 hours before samples are taken for plasma ammonia determination.[44] Dietary protein and the infusion of crystalline amino acids in solution are both known to increase plasma ammonia concentration.[45] Another possibility is environmental ammonia

intoxication. The recommended maximum allowed air ammonia concentration in the work place for man is 25 ppm (8 hr/day, 5 day/week). Rat cages frequently have concentrations above this level.[46]

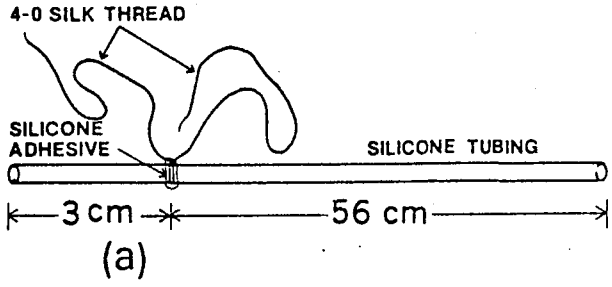
In an attempt to conserve animals, this experiment was designed so blood samples would be collected from each animal at each sampling time. In order to do this while minimizing the effects of repeated blood sampling, each sample was just large enough to permit one trial of each assay. Thus it was not possible to double check any values which seemed unusual. Each assay was also scaled down by a factor of ten to conserve blood in the animals. Such small samples may have decreased assay precision.

The objectives of this study were to evaluate the efficacy of a balanced amino acid infusion on recovery from temporary hepatic insufficiency, and their effect on plasma ammonia levels. While results of the LDH and bilirubin assays were inconclusive, the GPT assay suggests that the balanced formula was as effective as Freamine III in minimizing inflammation and necrosis of remaining hepatocytes after partial hepatectomy. Data from the ammonia assay suggests that the balanced formula was more effective than Freamine III in maintaining plasma ammonia levels within the normal range after partial hepatectomy. Difficulties with animal viability and cannula patency seen in the balanced formula group were no worse than seen with

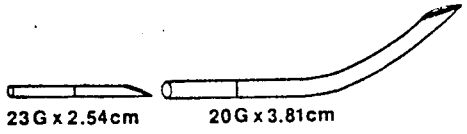
the electrolyte controls, suggesting that these problems were not a directly related to the balanced formula's amino acid composition. Unfortunately, the results of this study were not totally conclusive. More work needs to be done to fully evaluate the therapeutic potential of amino acid balanced solutions. Perhaps with a different experimental model, assays with greater sensitivity, and a cooperative team effort, it may be possible to fully validate this concept.

Figure 1a. Catheter used for infusing solutions into the jugular vein (modified from Bakar et.al.[26]).

Figure 1b-1d. Preparation of implant needle (see text for detail, from Bakar et.al.[26]).

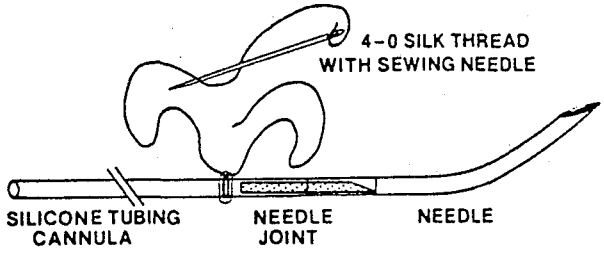

4-0 SILK THREAD
SILICONE ADHESIVE
SILICONE TUBING
3 cm
56 cm

(a)


23G x 2.54cm
20G x 3.81cm

(b)

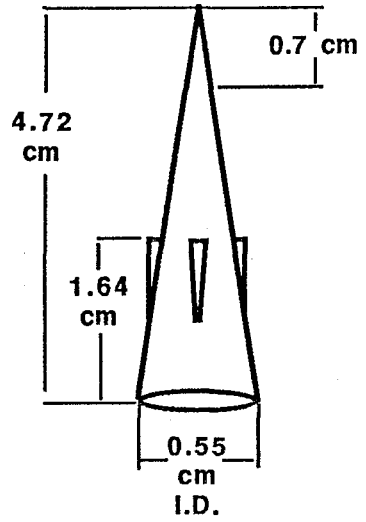
(c)

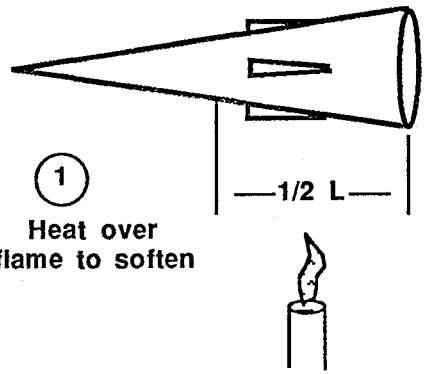

4-0 SILK THREAD
WITH SEWING NEEDLE
SILICONE TUBING
CANNULA
NEEDLE
JOINT
NEEDLE

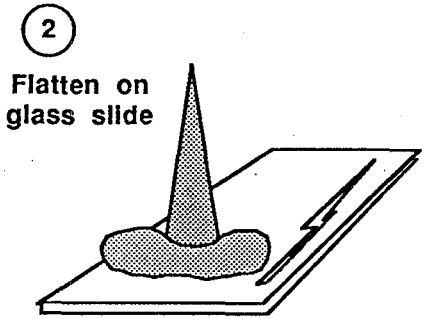
(d)

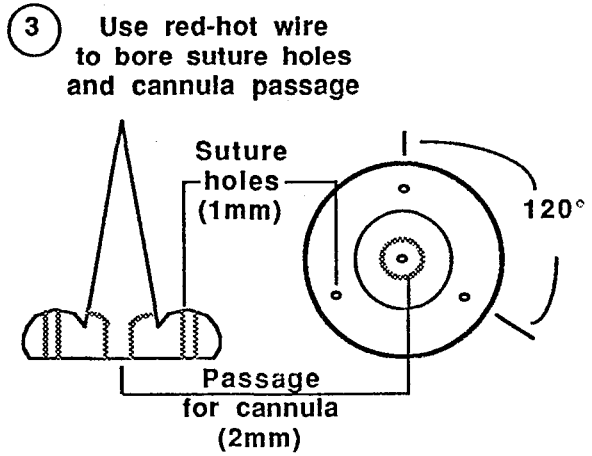
Figure 2. Fabrication of Implant Button (Model 1), Using a MLA 0 - 200 Microliter Polyethylene Pipette Tip.

Implant Button Model 1









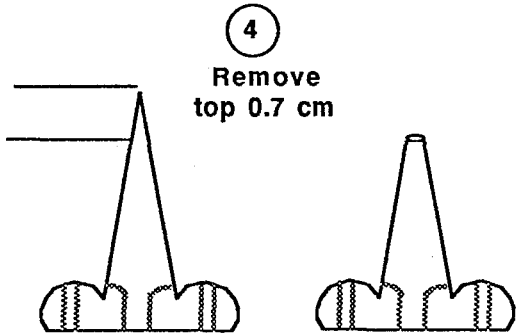
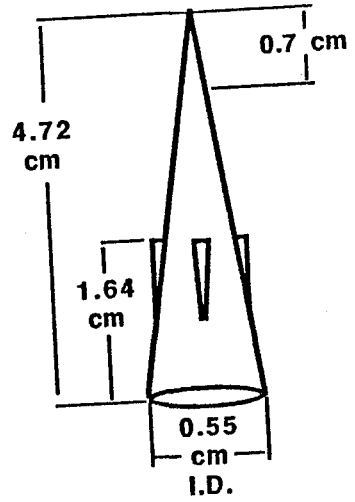


Figure 3. Fabrication of Implant Button (Model 2), Using a MLA 0 - 200 Microliter Polyethylene Pipette Tip.

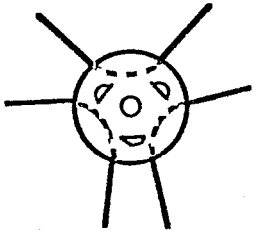
Implant Button Model 2



(1) Remove top 7 mm.

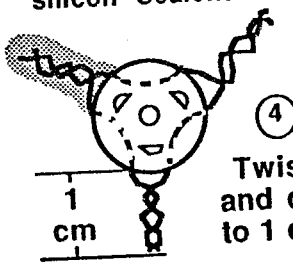
(2) 1 mm die holes on each side of projection

(3) Thread wires through holes



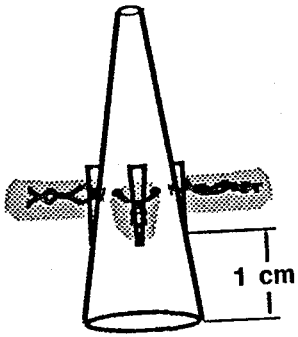
TOP VIEW

(5) Coat with silicon Sealent



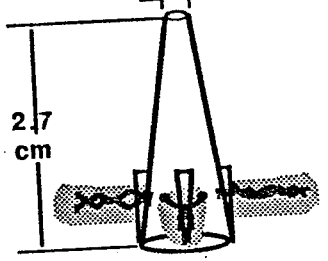
(4) Twist and cut to 1 cm

TOP VIEW



(6) Remove Bottom 1 cm

O.D.≅ 2.5 mm



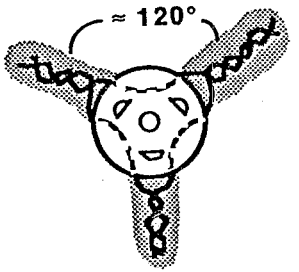
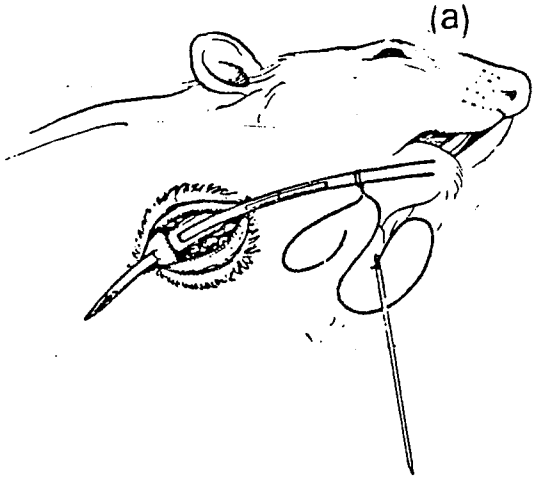
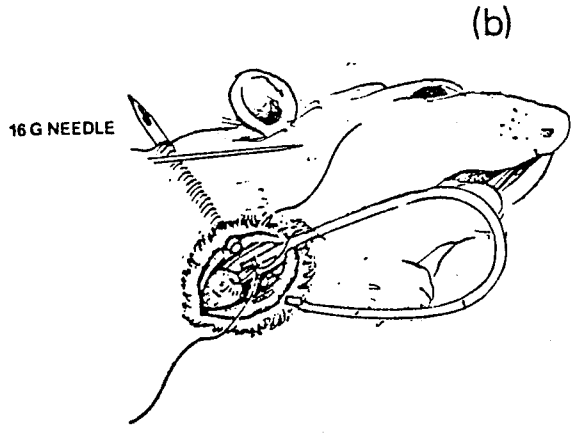


Figure 4a. Insertion of implant needle and catheter into jugular vein. Note that the needle is brought out through the deltoid muscle. (modified from Bakar[26]).

Figure 4b. Securing the catheter and tunneling under the skin (modified from Bakar[26]).

Figure 4c. Exteriorization of catheter and implant button. (from Bakar[26]).


(a)


(b)
16 G NEEDLE


(c)
STYLET
CANNULA
END

Figure 5. Cannulated animal and swivel assembly. Animal is shown with implant button. (Modified from King-Chatham[47]).

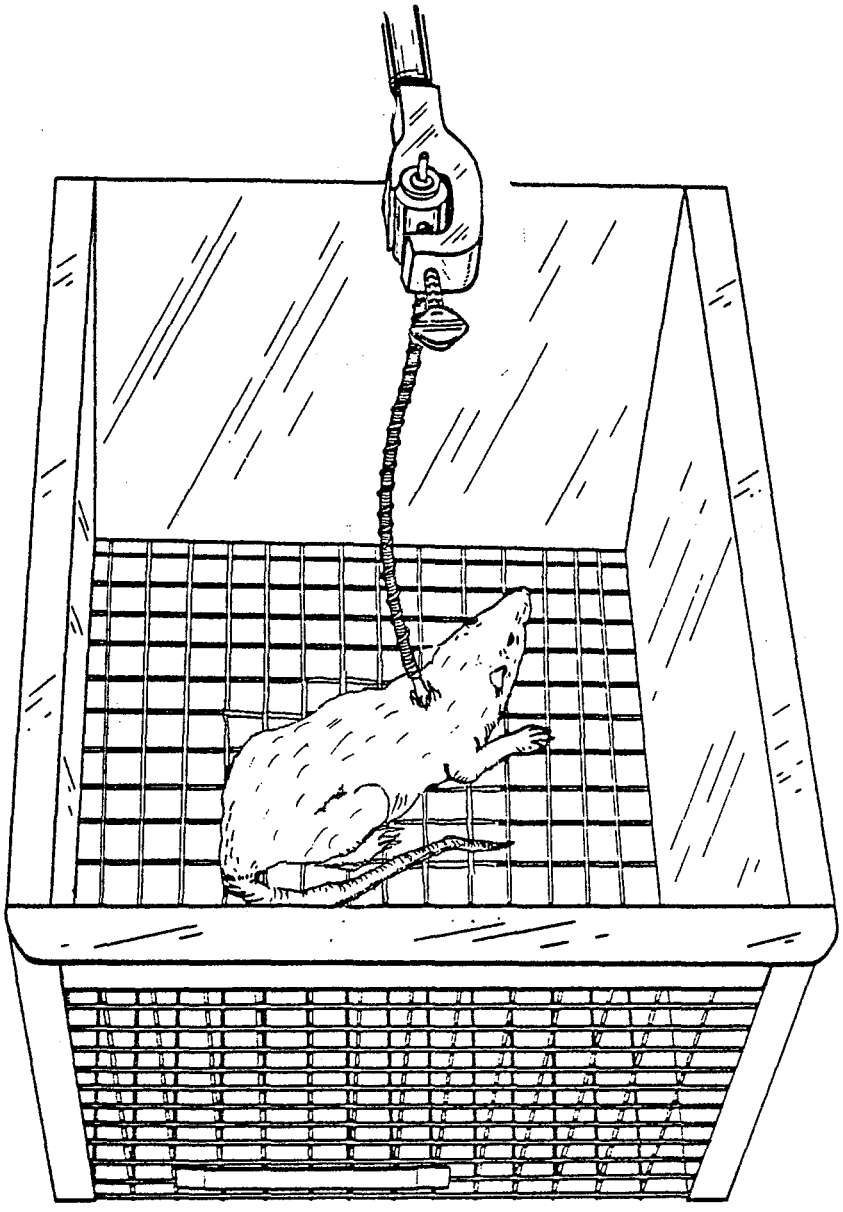


TABLE 1

Vitamins and Electrolytes Added
to Amino Acid Solutions

Viatmins	g/l
Choline	40.62
Folate	0.03
Niacin	0.6
Pantothenic acid	0.24
Riboflavin	0.09
Thiamin	0.14
B6	0.22
B12	0.002

Electrolytes	meq
Sodium	25
Potassium	20
Calcium	5
Magnesium	5
Chloride	30
Acetate	25
total mOsm/L	4200

TABLE 2

Amino Acid Concentration of Serum and 10% Balanced Formula

AMINO ACID	M.W.	serum(1) (nmol/ml)	serum (mg/1)	serum10% (mg/1)	Balanced (mg/1)
ILE	131.20	54.94	7.21	1633	1635
LEU	131.20	98.70	12.95	2934	2927
LYS	146.20	127.30	18.61	5274	5276
MET	149.20	48.05	7.17	1624	1622
PHE	165.20	40.80	6.74	1527	1530
THR	119.20	219.30	26.14	5923	5930
TRP	204.20	127.35	26.00	5892	5890
VAL	117.20	135.20	15.85	3590	3592
ALA	89.10	379.50	33.81	7661	7672
ARG	174.20	185.55	32.32	7324	7326
HIS	155.20	47.25	7.33	1662	1676
PRO	115.20	740.45	85.30	19327	19349
SER	105.20	200.30	21.07	4774	4792
TYR	181.20	66.05	11.97	2712	200
GLY	75.10	270.05	20.28	4595	4602
CYS-HCl	121.20	34.75	4.21	955	956
GLU	147.10	130.45	19.19	4348	4330
ASP	133.10	30.10	4.01	908	910
GLN	146.10	505.15	73.80	16722	16727
ASN	132.10	55.85	7.38	1901	1920

TABLE 3

Comparison of Freamine III and balanced formula amino acid composition

AMINO ACID	Balanced (mg/l)	FreamineI (mg/l)	Molar ratio (Leu based) serum	Balanced	FreamineIII
ILE	1635	6900	0.56	0.56	0.76
LEU	2927	9100	1.00	1.00	1.00
LYS	5276	7300	1.29	1.62	0.72
MET	1622	5300	0.49	0.49	0.51
PHE	1530	5600	0.41	0.42	0.49
THR	5930	4000	2.22	2.23	0.48
TRP	5890	1500	1.29	1.29	0.11
VAL	3592	6600	1.37	1.37	0.81
ALA	7672	7100	3.84	3.86	1.15
ARG	7326	9500	1.88	1.88	0.79
HIS	1676	2800	0.48	0.48	0.26
PRO	19349	11200	7.50	7.53	1.40
SER	4792	5900	2.03	2.04	0.81
TYR	200		0.67	0.01	0.00
GLY	4602	14000	2.74	2.75	2.69
CYS-HCl	956	240	0.35	0.35	0.03
GLU	4330		1.32	1.32	0.00
ASP	910		0.30	0.31	0.00
GLN	16727		5.12	5.13	0.00
ASN	1920		0.57	0.65	0.00

Figure 6. Partial Hepatectomy Proceedure

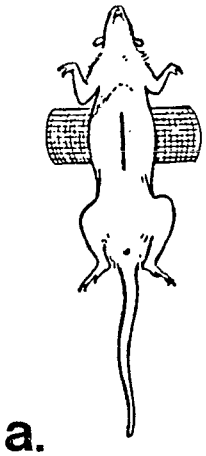
a. abdominal insicion

b. "poping" the liver (medial and left lateral lobes) out of the abdominal cavity.

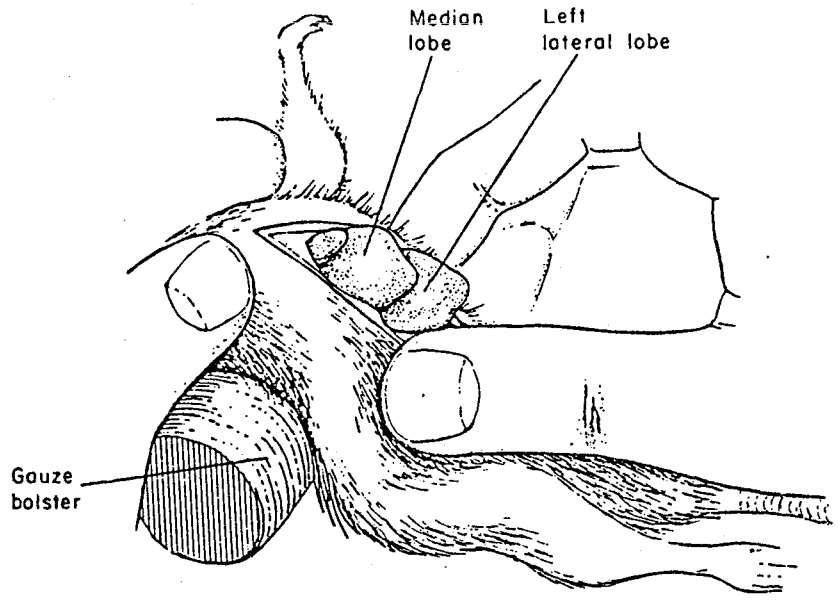
c. suspensory ligaments cut during the procedure.

d. ligature placement.

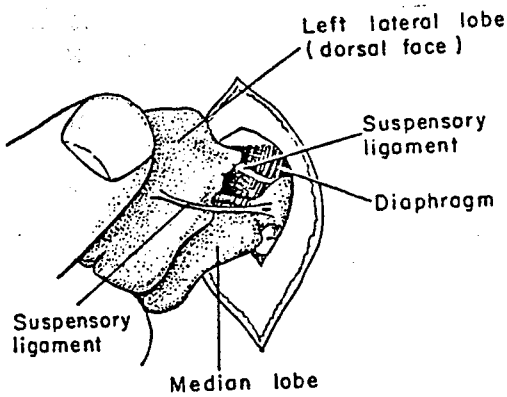
e. preperation for liver transsection (all from Waynforth[27]).



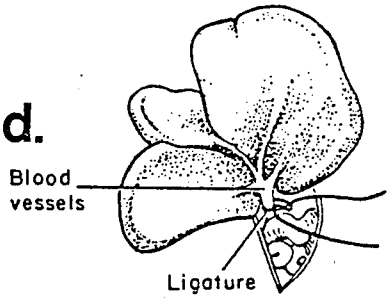
a.


Median lobe
Left lateral lobe
Gauze bolster

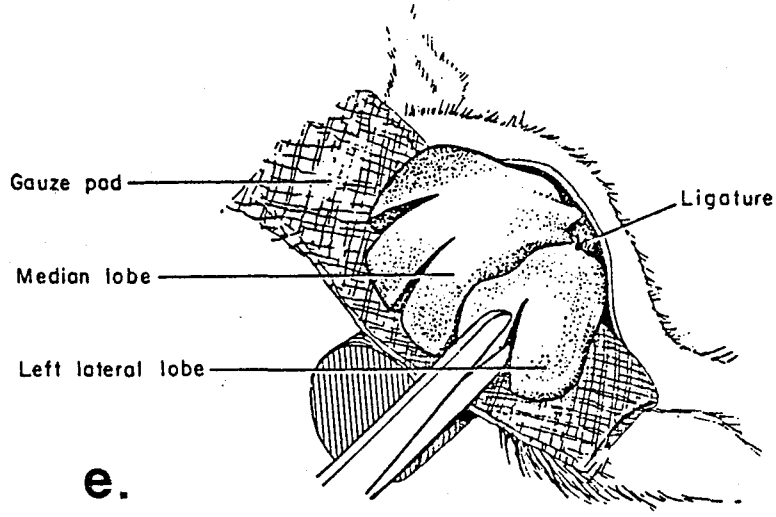
b.


Left lateral lobe (dorsal face)
Suspensory ligament
Diaphragm
Suspensory ligament
Median lobe (dorsal face)

c.


Blood vessels
Ligature

d.


Gauze pad
Median lobe
Left lateral lobe
Ligature

e.

TABLE 4

Group designation and variables.

Group	Surgery	Infusion (1).	Food (2).
IA. (Saline)	Part. Hep.	Half Normal Saline	Rat Chow
IB. (Electrolyte)	Part. Hep.	Hyperlyte Electrolytes	Rat Chow
II. (Freamine)	Part. Hep.	Freamine III Amino acids	Glucose Pellets
III. (Balanced)	Part. Hep.	Balanced Form. Amino acids	Glucose Pellets
IV. (Sham)	Sham	Hyperlyte Electrolytes	Rat Chow

(1). Infusion rate for all solutions was 30 mL/day.

(2). All food was provided ad libitum.

Figure 7. Standard Curve for Lactate Dehydrogenase Assay
Each point is based on nine determinations.

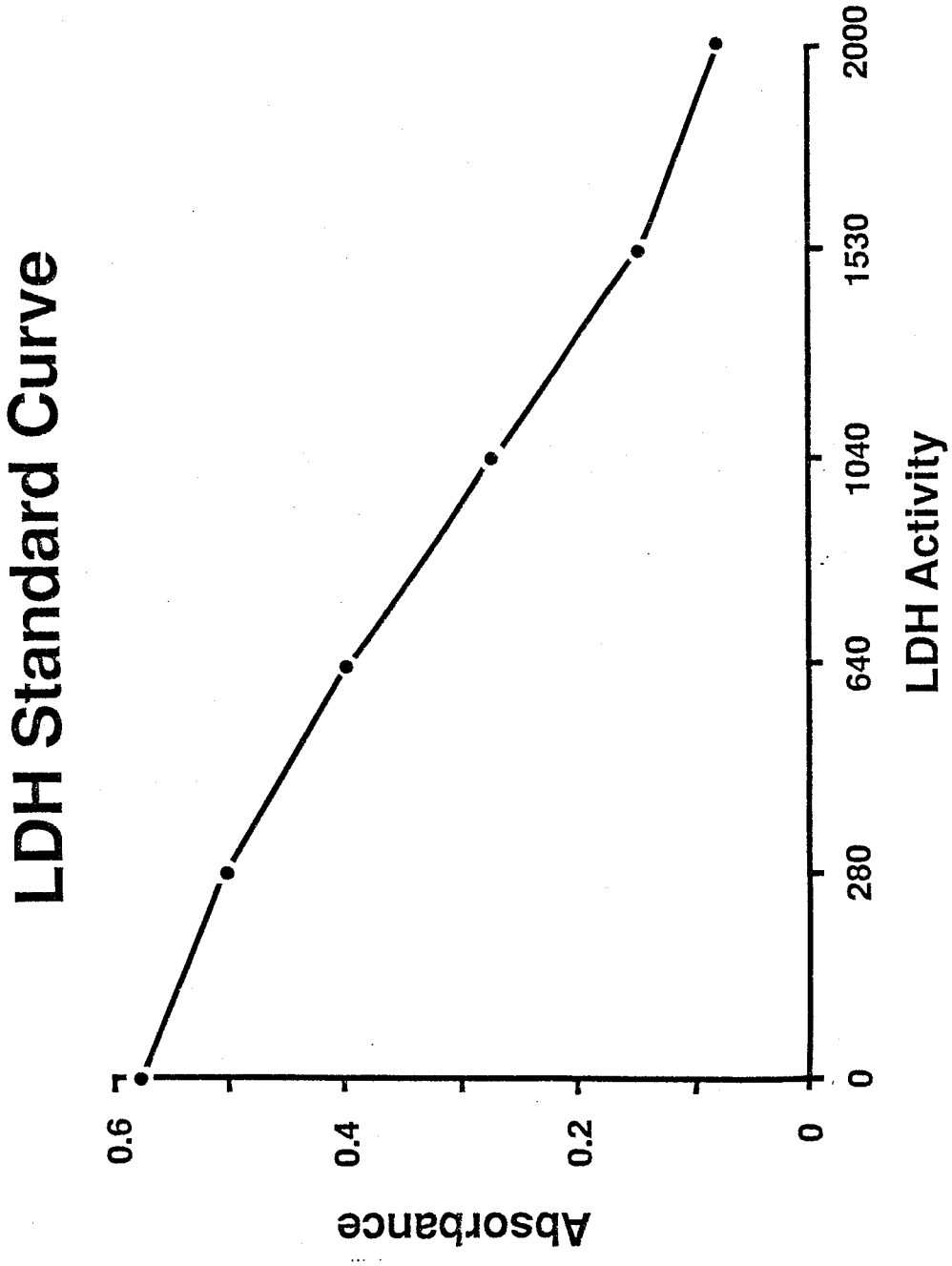

LDH Standard Curve
Absorbance
0.6
0.4
0.2
0
0
280
640
1040
1530
2000
LDH Activity

Table 5

LDH Standard Curve Tube Contents

tube #	Pyruvate	water	color reagent	LDH act.
1	100	10	100	0
2	80	30	100	280
3	60	50	100	640
4	40	70	100	1040
5	20	90	100	1530
6	10	100	100	2000

Notes:
1. Values listed for pyruvate, water and color reagent are microliters
2. 20 min. after mixing the above reagents, 1.0 ml of 0.40 N NaOH was added to each tube
3. LDH activity is in Berger-Broida units per ml (1 IU LDH activity = 0.48 B-B unit/ml)

TABLE 6

Bilirubin assay tube contents

component	plasma blank	total	direct
serum	20	20	20
HCl	50	0	100
Caffeine	100	100	0
Diazo reagent	0	50	50
Cysteine	10	10	10
Alkaline Tartrate	150	150	150

Notes: 1. Above values are microliters

2. Concentrations of reagents are listed under methods.

Figure 8. GPT Standard Curve
Each Point is based
on nine determinations

GPT Standard Curve

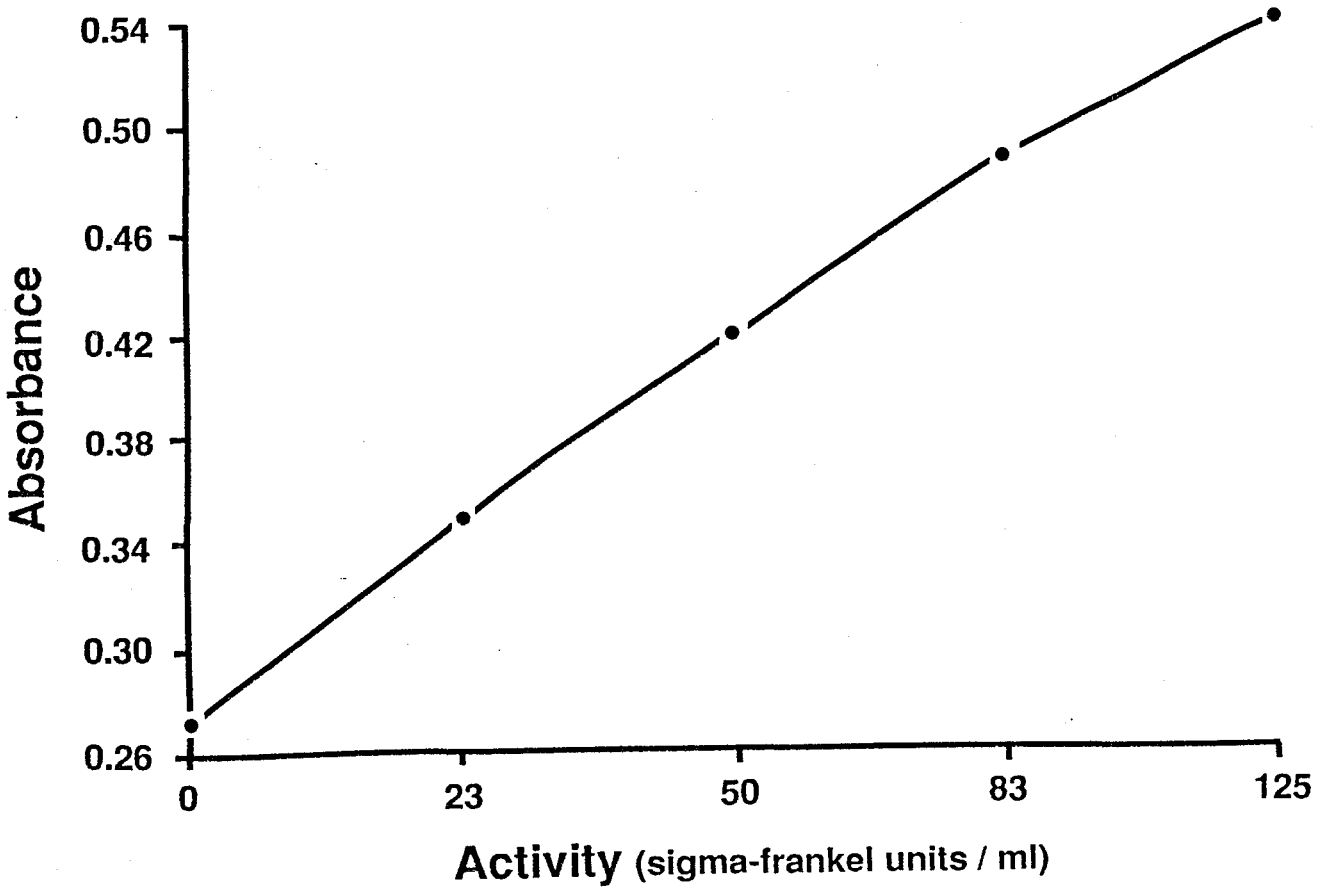


TABLE 7

GPT Standard Curve Tube Contents

tube #	Calibration standard	substrate	water	GPT activity
1	0	100	20	0
2	10	90	20	23
3	20	80	20	50
4	30	70	20	83
5	40	60	20	125

Notes:

1. 100 microliters of Sigma color reagent was added to each tube after the above reagents were mixed together.
2. 20 min after adding the color reagent, 1.0 ml of 0.4 N NaOH was added to each tube.
3. Reagent volumes listed are microliters
4. Activity is in Sigma-Frankel units/ml (1 IU GPT activity = 0.48 S-F unit/ml).

TABLE 8

Animal viability

group	Suvival Time avg	s.d.	var
Freamine III	14.00	0.00	0.00
Balanced	11.00	2.76	7.62
Electrolyte	8.67	2.05	4.20
Sham	12.67	1.89	3.57

Probable Cause of Death

	a	b	c	d	e
Freamine III	60%	20%			20%
Balanced	40%	40%	20%		
Electrolyte		60%			40%
Sham	20%	20%		60%	

key

a. finished the experiment
b. thrombus
c. hepatic failure
d. terminated due to sampling problems
e. accidental death

Weight Change

Group	Cannulation Weight	Final Weight	Change	Percent Change
Freamine	140.60	142.00	1.40	101.00
Balanced	138.20	139.50	1.25	100.94
Electrolyte	159.00	141.50	-17.00	88.99
Sham	155.33	148.67	-6.67	95.71

Figure 9. LDH activity after partial hepatectomy in saline control animals. Results are averages of five animals. A triplicate determination was made for each animal. Dashed line represents 0 activity, and activity increases as absorbance decreases.

LDH Assay

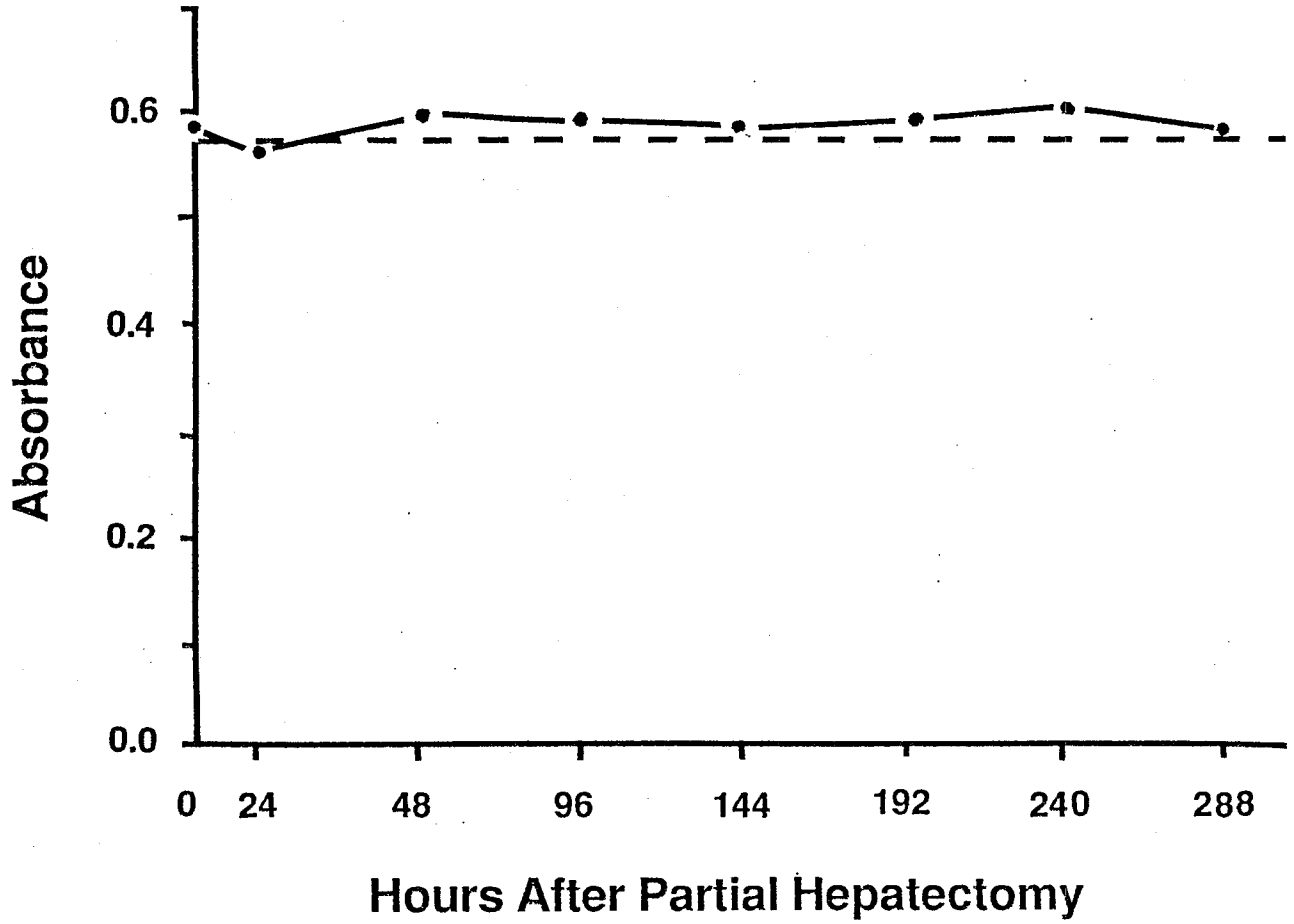

Absorbance
0.6
0.4
0.2
0.0
0
24
48
96
144
192
240
288
Hours After Partial Hepatectomy

Figure 10. Hematocrit values after partial hepatectomy. 0.2 ml of blood was removed at each sampling time. Results are expressed as a percentage of presurgery values and are averages of all animals within each group.

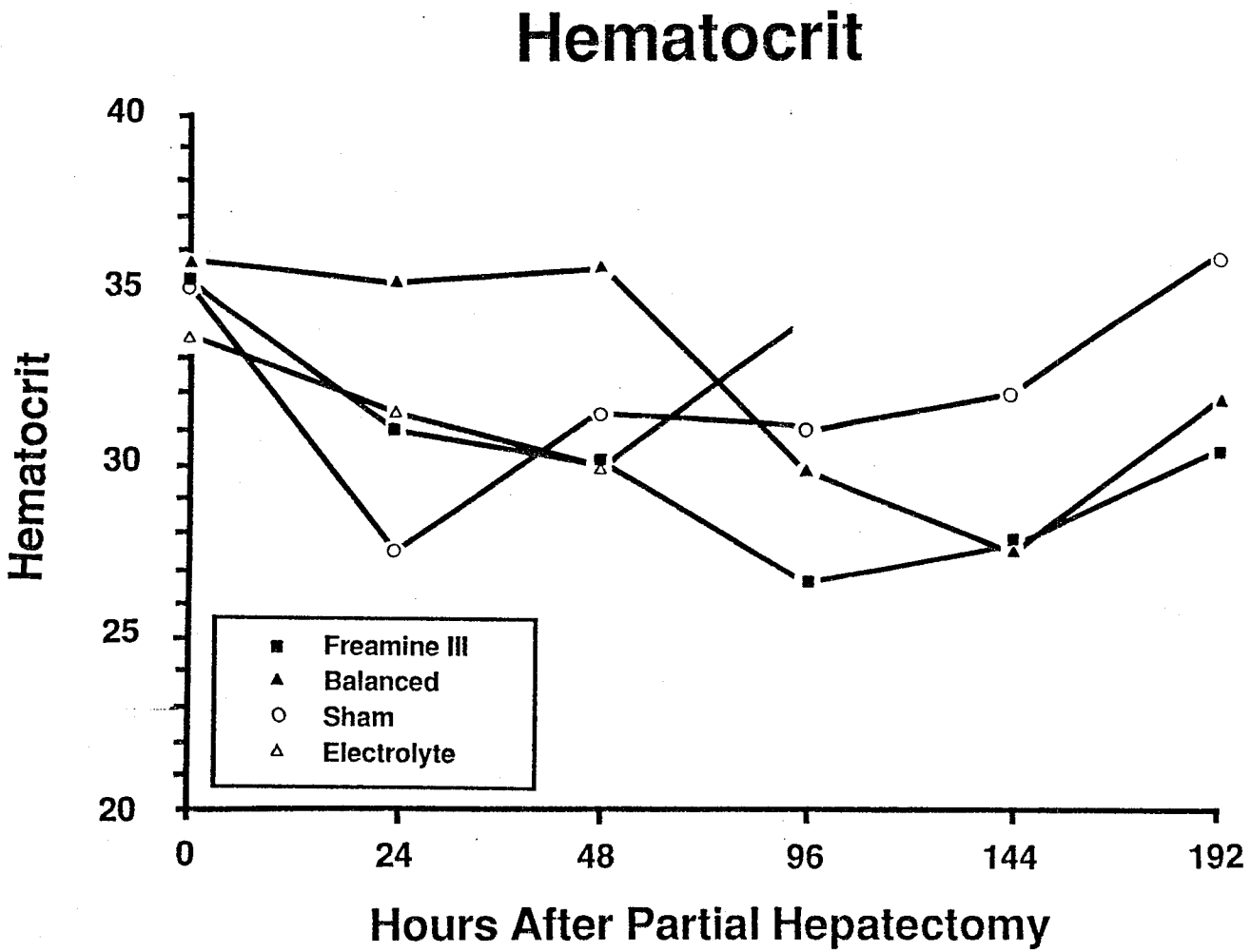

Hematocrit
40
35
30
25
20
Hematocrit
Freamine III
Balanced
Sham
Electrolyte
0
24
48
96
144
192
Hours After Partial Hepatectomy

Figure 11. Direct plasma bilirubin levels after partial hepatectomy. Group values are averages of single determinations from each animal.

Direct Plasma Bilirubin

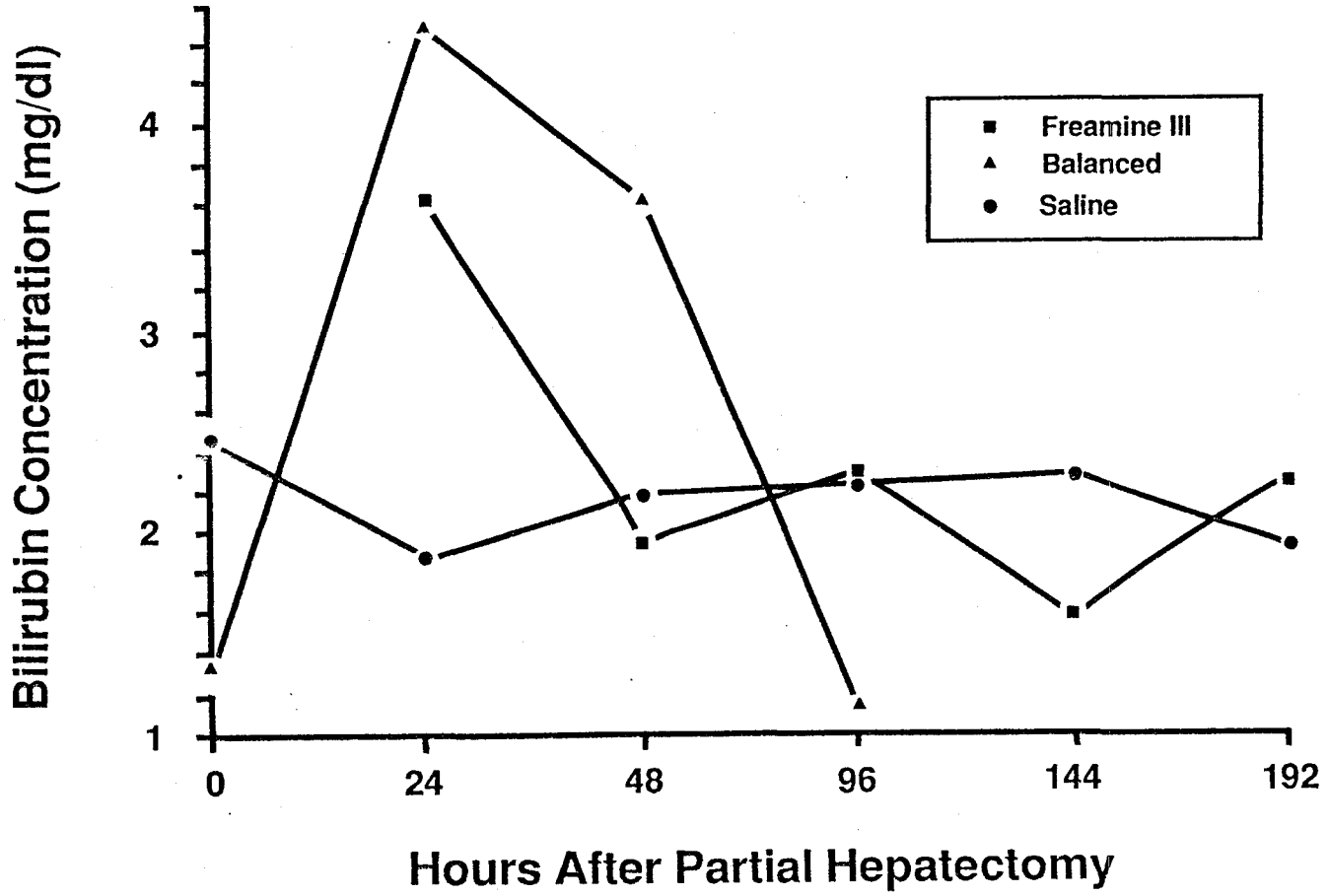


TABLE 9

Direct Plasma Bilirubin

Group		0 hr	24 hr	48 hr	96 hr	144 hr	144 hr	192 hr
Freamine III	avg.		3.64	1.93 *	2.28	1.58	1.58	2.24
	s.d.		1.04	0.5	0.32	0.09	0.09	0.61
	n		4	4	3	2	2	2
Balanced	Avg.	1.35	4.49	3.63	1.14			
	s.d.	0.47		1.73	0.22			
	n	4	1	5	3			
Saline	avg.	2.46	1.87	2.17 **	2.22 **	2.27	2.27	1.92
	s.d.	0.17	0.47	1.02	0	0.09	0.09	0.5
	n	4	4	4	2	2	2	2
Sham	avg.	0.25	0.34					
	s.d.	0.01	0.07					
	n	3	2					

* $p < 0.1$ ** $p < 0.05$ *** $p < 0.025$

Figure 12. Total plasma bilirubin levels after partial hepatectomy. Group values are averages of single determinations from each animal.

Total Plasma Bilirubin

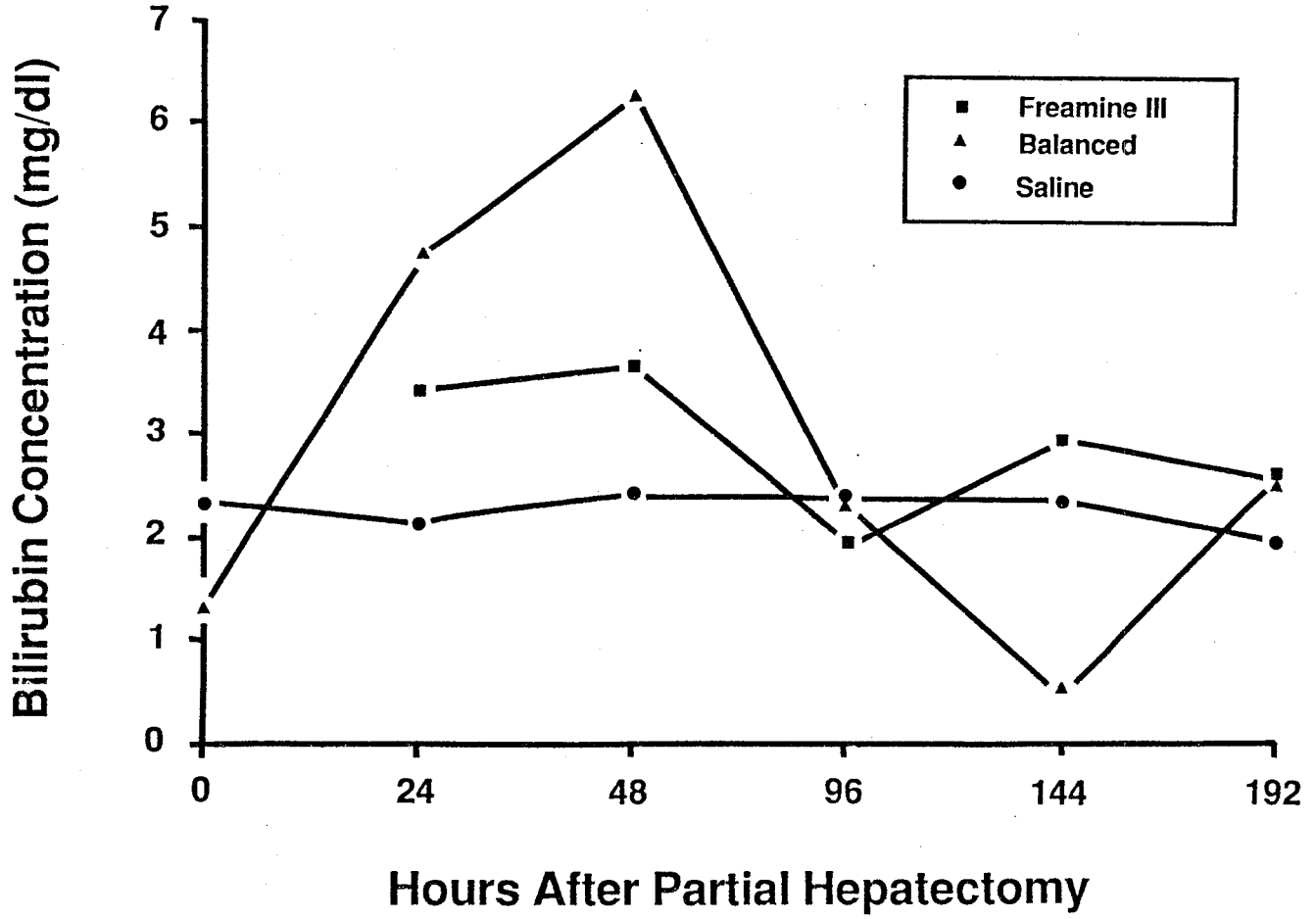


TABLE 10

Total Plasma Bilirubin

Group		0 hr	24 hr	48 hr	96 hr	144 hr	192 hr
Freamine III	avg.		3.42	3.66	1.94	2.95	2.58
	s.d.		0.77	0.36	0.08	0.04	0.27
	n		4	3	4	2	2
Balanced	avg.	1.28	4.7	6.25	2.29	0.49	2.54
	s.d.	0.56	2.89	3.43	0.38	0.07	0.37
	n	54	5	5	4	2	2
Saline	avg.	2.29	2.13	2.43	2.35	2.4	1.96
	s.d.	0.05	0.42	1	0.09	0.01	0.34
	n	3	4	4	3	3	2
Sham	avg.	0.33	1.02	0.22			
	s.d.	0.03	0.88	0			
	n	3	2	2			

Figure 13. Plasma ammonia concentrations. Group values are averages of single determinations from each animal.

Plasma Ammonia Levels

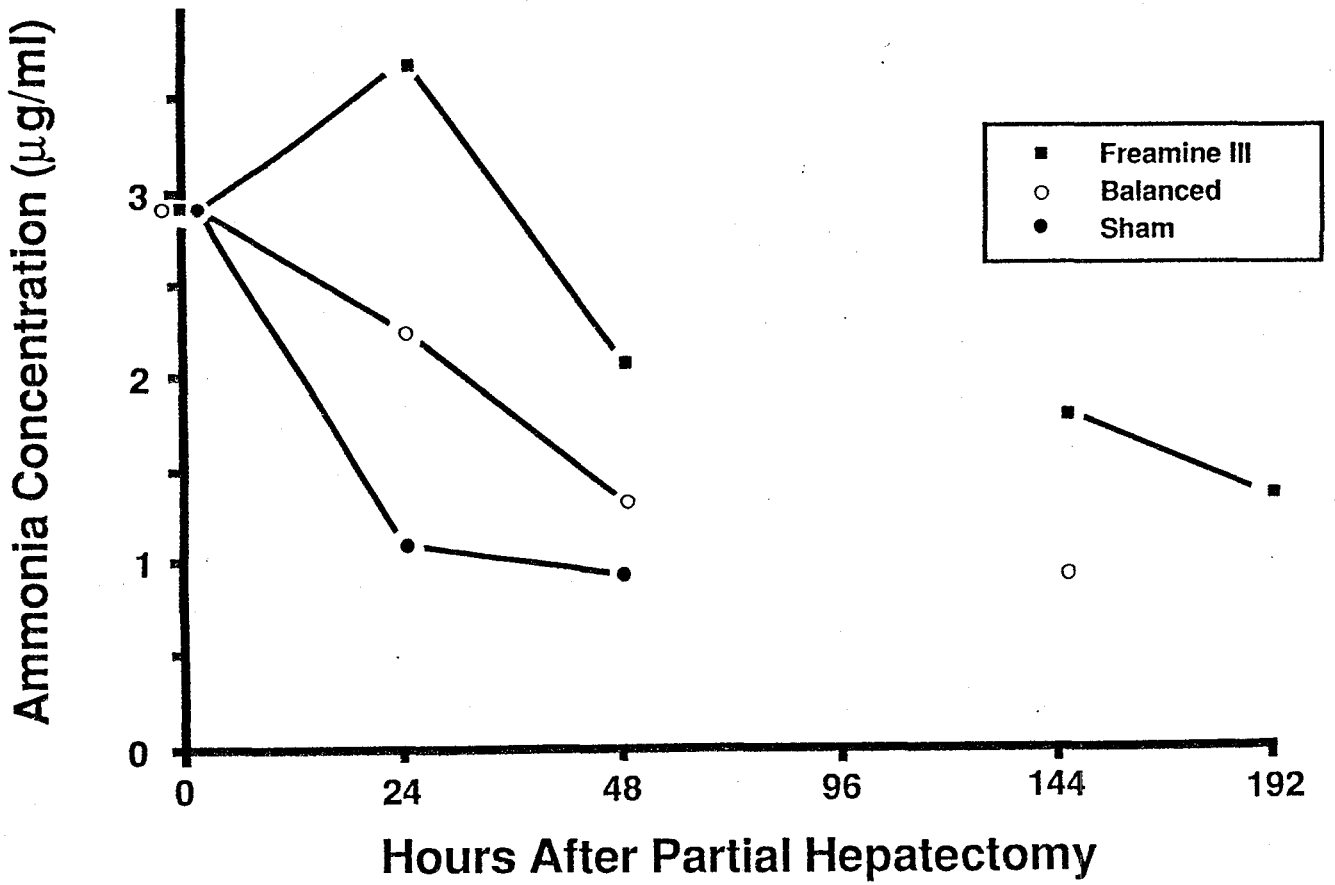


TABLE 11

Plasma GPT Activity

Group		0 hr	24 hr	48 hr	96 hr	144 hr	192 hr
Freamine III	avg.	31.56	108.51 *	51.82*	11.35***	-2.36	7.83
	s.d.	2.33	24.19	16.12	4.05	2.36	7.25
	n	4	4	5	4	1	2
Balanced	Avg.	27.3	116.54**	64.18	28.21	15.13 *	8.75
	s.d.	0.84	16.36	16.49	3.87	10.87	5.91
	n	4	4	4	3	2	2
Saline	avg.	29.3	181.18	102.02	41.76	36.19	34.04
	s.d.	2.49	13.13	7.54	12.44	1.05	2.05
	n	3	2	2	1	3	1
Sham	avg.		79.9	68.55			
	s.d.		1.89	2.36			
	n		2	2			

* $p < 0.1$ ** $p < 0.05$ *** $p < 0.025$

Figure 14. Plasma glutamic pyruvic tranaminase activity after partial hepatectomy. Group values are averages of single determinations from each animal.

GPT Activity

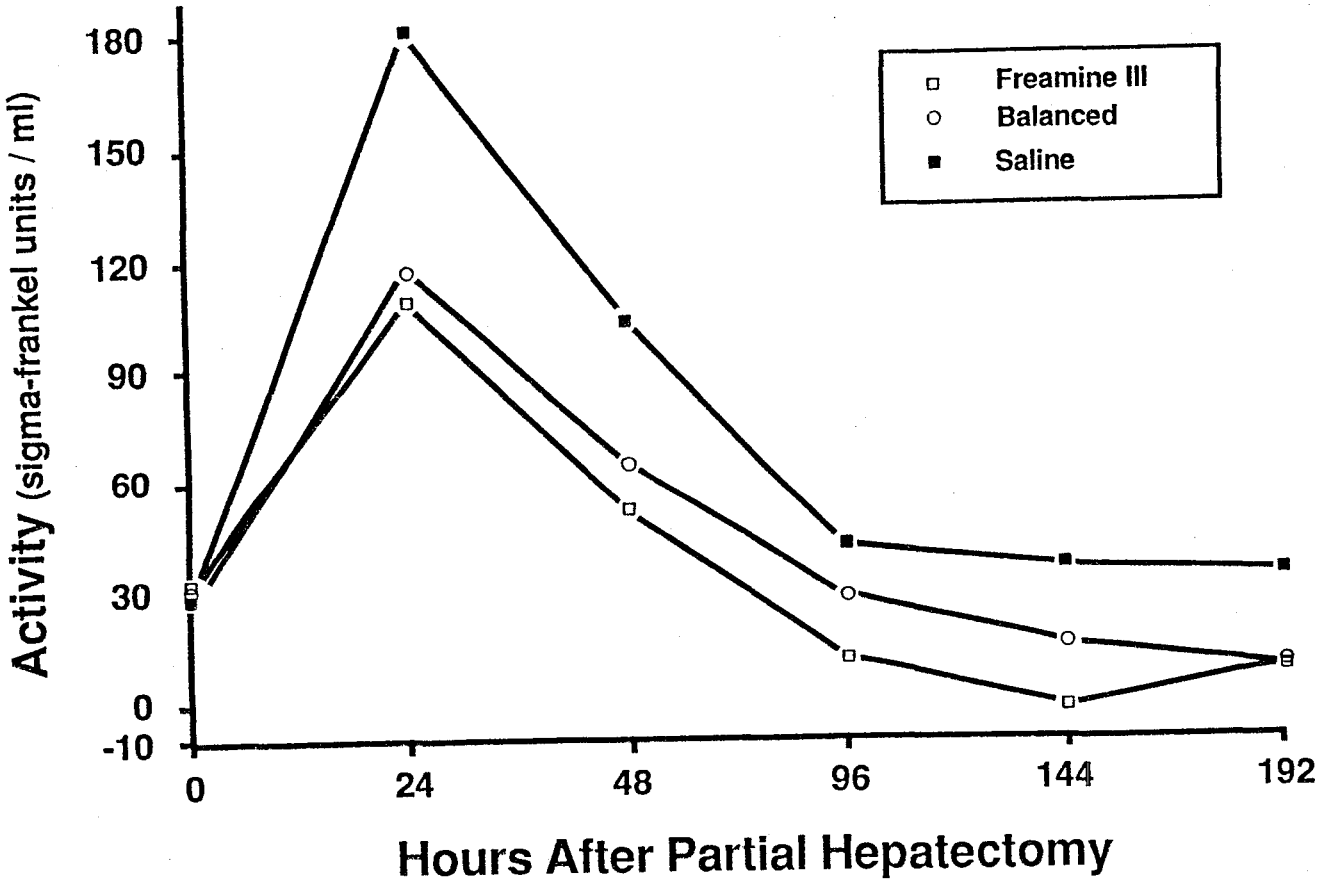
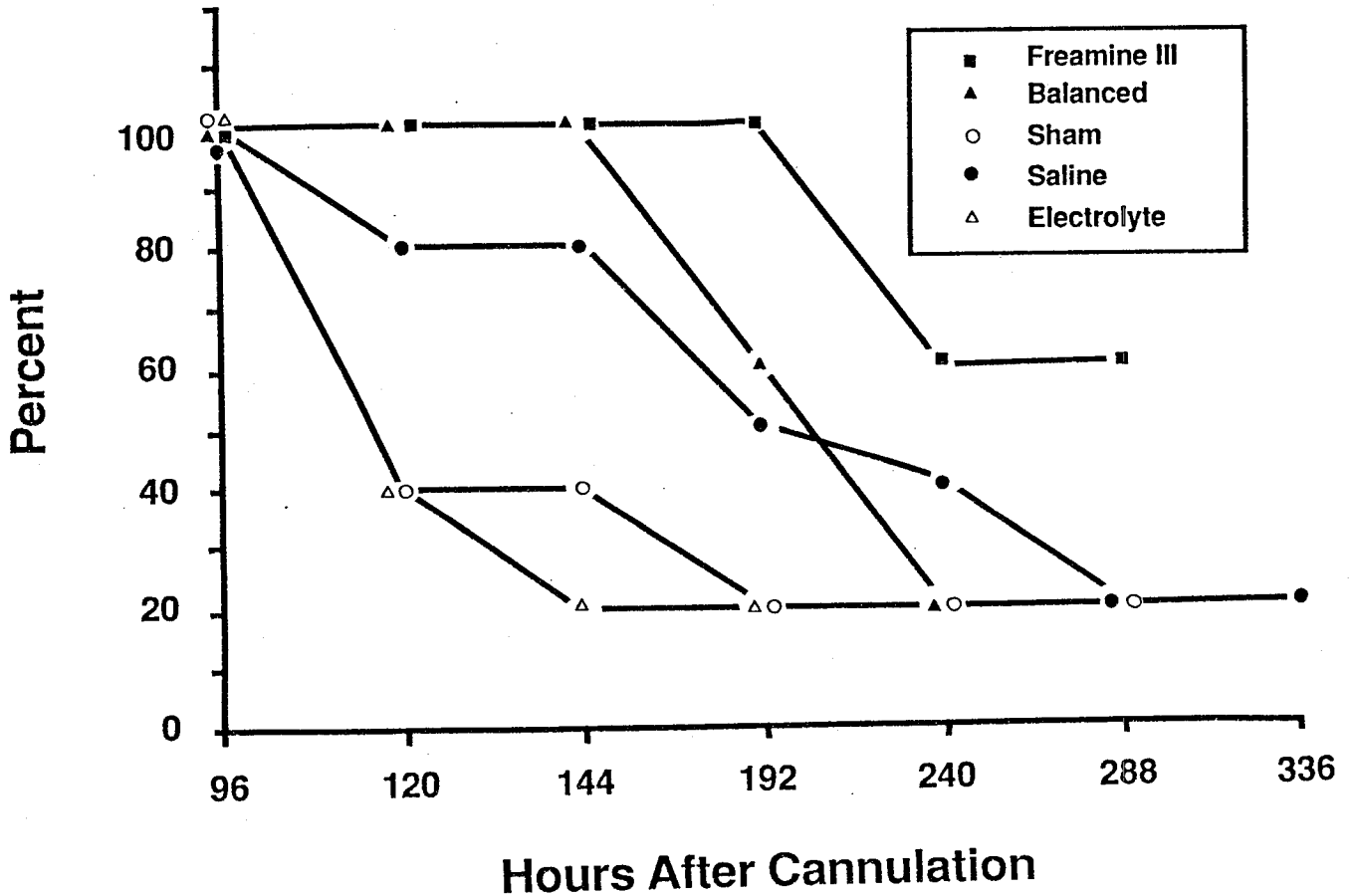


Figure 15. Cannula Patency
Patency is used here as the ability to obtain blood samples from an animal. Percentages in this graph are bases on the total number of animals starting in each group. Thus patency decreased with cannula occlusion and/or animal death.

Cannula Patency



References

1. Lehninger, A.L., Principles of Biochemistry. Worth Publishers Inc. New York, 1982.

2. Coon, W.W., and Kowalczyk, R.S. In Manual of Surgical Nutrition. Ed. by W.F. Ballinger et.al., W.B. Saunders Co., Philadelphia, 1975.

3. Berry, H.K. In Newer Methods of Nutritional Biochemistry. v. 4, Ed. by Albanese, A.A., Academic Press, New York, 1970, pp. 79-122.

4. Maiz, A., Moldawer, L.L., Bistran, B.R., Birkhahn, R.H., Long, C.L., and Blackburn, G.L. Biochem. J., 226:43-50, 1985.

5. Moore, F.D., and Brennen, M.B. In Manual of Surgical Nutrition. Ed. by W.F. Ballinger et.al., W.B. Saunders Co., Philadelphia, 1975.

6. Moldawer, L.L., O'Keefe, J.D., Bothe, A., Bistran, B.R., and Blackburn, G.L. Metabolism , 29(2):173-180, 1980.

7. Long, C.L., Birkham, R.H., Geiger, A.B., and Blakemore W.S., The American Journal of Clinical Nutrition 34:1087-1093, 1981.

8. Barton, R.N. British Medical Bulletin., 41(3):218-225, 1985.

9. Blackburn, G.L., Flatt, J.P., Clowes, G.H.A., O'Donnel, T.F., and Hensle, T.E. Ann. Surg., 177(5):588-594, 1973.

10. Freund, H.R., James, J.H., and Fischer, J.E. Surgery, 90(2):237-243, 1981.

11. Sakamoto, A., Moldawer, L.L., Palombo, J.D., Desai, S.P., Bistran, B.R., and Blackburn, G.L. Clin. Sci., 64:321-331, 1983.

12. Skillman, J.J., Rosenoer, V.M., Pallotta, J.A., Young, J.B., Young, V.R., Long, P.C., Wilentz, K. and Munro, H.N., Surgery, 89(2):168-174, 1981.

13. Freund, H., Yoshomura, N., and Fischer, J.E. J Surg. Res., 29(1):23-30, 1980.

14. Buse, M.G., Jursinic, S., and Reid, S.S., Biochem. J. 148:363-374, 1975.

15. Freund, H., Yosomura, N., Lunetta, L., and Fischer, J.E. Surgery, 83(6):611-618, 1978.

16. Ohashi, H., Takami, T., and Yugari, Y. Nutr. Rep Int. 33(5):739-745, 1886.

17. Sakamoto, A., Moldawer, L.L., Bothe, A., Bistran, B.R., and Blackburn, G.L. Surgical Forum, 31:99-100, 1980.

18. Sakamoto, A., Moldawer, L.L., Usui, S., Bothe, A., Bistran, B.R., and Blackburn, G.L. Surgical Forum, 30:67-69, 1979.

19. Desai, S.P., Bistran, B.R., Moldawer, L.L., Miller, M.M., and Blackburn, G.L. J. Trauma, 22(9):747-752, 1982.

20. De Caterina, M., Piccolboni, D., Alfieri, R., Spagnuolo, G., Petre, C., Aliperti, G., Del Gaudio, F., and Rendano, F., La Ricerca Clin. Lab., 15:79-88, 1985.

21. Garlick, P.J., Millward, D.J., and James, W.P.T., Biochem J., 136:935-945, 1973.

22. McGowan, J.P. "Regulation of Protein Synthesis by Leucine and Amino Acid Balance". (Ph.D. diss., Univ. of the Pacific., 1982), p.5.

23. Sayre, F.W., Hansen, L., and Yarwood, E.A. Exper. Parisitol., 13:98, 1963.

24. Morgan, H.E., Jefferson, L.S., Wolpert, E.B., and Rannels, D.E. J. Biol. Chem., 246(7):2163-2170, 1971.

25. Grazer, R.E., Sutton, J.M., Freidstrom, S., and McBarron, F.D. Arch. Int. Med., 144:2279-2279, 1984.

26. Bakar, S.K., and Niazi, S. J. Pharm. Sci., 72(9):1027-1029, 1983.

27. Wayneforth, H.B. Experimental and Surgical Technique in the Rat., Academic Press, New York, 1980.

28. Steiger, E., Vars, H.M., and Dudrick, S.J., Arch. Surg., 104:330-332, 1972.

29. Upton, R.A. J. Pharm. Sci., 64(1):112-114, 1975.

30. Birkhahn, R.H., long, C.L., Fitkin, D., and Blakemore, W.S., J. Parent. and Ent Nutr., 3(6):421-423, 1979.

31. Yoburn, B.C., Morales, R., and Inturrisi, C.E. Physiol. and Behavior, 33:89-94, 1984.

32. Soto-Velesco, J.M., Steiger, E., Rombeau, J.L., Jagelman, D.G., and Srp, F. Clev. Clin. Quart., 51:159-166, 1984.

33. Bauer, J.D. Clinical Laboratory Methods , The C.V. Mosby Co., St. Louis, 1982.

34. Ringler, D.H., and Dabich, L. In The Laboratory Rat, v.1 Ed. by H.J. Baker et.al. Academic Press, New York, 1979.

35. Merck Manual, 14th ed. Ed. by R. Berkow, Merck Sharp and Dohme, Rahway, 1982.

36. Cutler, M. Toxicol. and App. Pharmacol., 28:349-357, 1974.

37. Weinbren, K., and Billing, B.H. Brit. J. Exptl. Path., 30:199-204, 1956.

38. Zeive, L., Anderson, W.R., Lindblad, S. J. Lab. Clin. Med., 105(3):331-336, 1985.

39. Gartner, U., Stockert, R.J., Morell, A.G., and Wolkoff, A.W., Hepatology, 1(2):99-106, 1982.

40. Wood, C.B., Karran, S.J., and Blumgart, L.H. Brit. J. Surg., 60(8):613-617, 1973.

41. Kahn, D., Stadler, J., Terblanche, J., and Van Hoorn-Hickman, R. Gastroenterology, 79(5):907-911, 1980.

42. Sigma Technical Bulletin No. 505, Sigma Chemical Co., St. Louis, 1982.

43. Cohn, R.M., and Roth, K.S. Metabolic Disease, W.B. Saunders Co., Philadelphia, 1983.

44. Sigma Ammonia Procedure No. 170-UV, Sigma Chemical Co., St Louis, 1983.

45. Heird, W.C., MacMillan, R.W., and Winters, R.W. In Total Parenteral Nutrition, Ed. by J.E. Fischer, Little, Brown and Co., Boston, 1976.

46. Baker, H.J., Lindsey, R., and Weisbroth, S.H. In The Laboratory Rat, Ed, by H.J. Baker et.al., Academic Press Inc., New York, 1979.

47. Alice King Chatham Medical Arts Product Catalog. Los Angeles, CA

48. Partsch, G. and Adamiker, D. Z. Versuchstierk. 25:69-72, 1983.